



## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

<b>(51) International Patent Classification<sup>4</sup> :</b> C07C 101/26, C07D 285/00 C07D 273/00, A61K 49/00 A61K 31/195, 31/555	<b>A1</b>	<b>(11) International Publication Number:</b> WO 89/ 00557  <b>(43) International Publication Date:</b> 26 January 1989 (26.01.89)
<b>(21) International Application Number:</b> PCT/GB88/00572 <b>(22) International Filing Date:</b> 15 July 1988 (15.07.88) <b>(31) Priority Application Numbers:</b> 8716778 8716914 <b>(32) Priority Dates:</b> 16 July 1987 (16.07.87) 17 July 1987 (17.07.87) <b>(33) Priority Country:</b> GB <b>(71) Applicant (for GB only):</b> COCKBAIN, Julian, Roderick, Michaelson [GB/GB]; 27 Ladbroke Road, London W11 3PD (GB). <b>(71) Applicant (for all designated States except GB US):</b> NY-COMED AS [NO/NO]; Nycoveien 1-2, N-0401 Oslo 4 (NO). <b>(72) Inventors; and</b> <b>(75) Inventors/Applicants (for US only) :</b> BERG, Arne [NO/NO]; Stasjonsveien 37D, N-1310 Blommenholm (NO); ALMÉN, Torsten [SE/SE]; Ynglingegatan 7B, S-217 74 Malmö (SE). KLAVENESS, Jo [NO/NO]; Skøyen Terrasse 15, N-0276 Oslo 2 (NO). RONGVED, Pål [NO/NO];		<b>Hovdens vei 13, N-1454 Hellvik (NO). THOMASSEN, Terje [NO/NO];</b> Meklenborglia 12, N-1273 Oslo 12 (NO).  <b>(74) Common Representatives:</b> COCKBAIN, Julian, R., M. et al.; Frank B Dehn & Co, Imperial House, 15-19 Kingsway, London WC2B 6UZ (GB).  <b>(81) Designated States:</b> AU, DK, FI, GB, HU, JP, NO, SU, US.  <b>Published</b> <i>With international search report.          Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i>
<b>(54) Title:</b> AMINOPOLYCARBOXYLIC ACIDS AND DERIVATIVES THEREOF  <b>(57) Abstract</b>  <p>There are provided chelating agents particularly useful for the preparations of diagnostic and therapeutic agents for magnetic resonance imaging, scintigraphy, ultrasound imaging, radiotherapy and heavy metal detoxification, said agents being compounds of formula <math>X-CHR_1-NZ-(CHR_1)_n-A-(CHR_1)_m-NZ-CHR_1-X</math> wherein (each of the groups Z is a group <math>-CHR_1X</math> or the groups Z together are a group <math>-(CHR_1)_q-A'-(CHR_1)_r</math>, where <math>A'</math> is an oxygen or sulphur atom or a group <math>-N-Y</math>; <math>A</math> is a group <math>-N-Y</math> or <math>A-(CHR_1)_m</math> represents a carbon-nitrogen bond or, when the groups Z together are a group <math>-(CHR_1)_q-A'-(CHR_1)_r</math>, <math>A</math> may also represent an oxygen or sulphur atom; each Y, which may be the same or different, is a group <math>-(CHR_1)_p-N(CHR_1X)_2</math> or a group <math>-CHR_1X</math>; each X, which may be the same or different, is a carboxyl group or a derivative thereof or a group <math>R_1</math>; each <math>R_1</math>, which may be the same or different, is a hydrogen atom, a hydroxyalkyl group or an optionally hydroxylated alkoxy or alkoxyalkyl group; n, m, p, q and r are each 2, 3 or 4; with the provisos that at least two nitrogens carry a <math>-CHR_1X</math> moiety wherein X is a carboxyl group or a derivative thereof, that each <math>-CHR_1X</math> moiety is other than a methyl group, and that unless <math>A'</math> is oxygen or sulphur or <math>A</math> is <math>N-(CHR_1)_p-N(CHR_1X)_2</math> at least one <math>R_1</math> is other than hydrogen) and salts thereof.</p>		

BEST AVAILABLE COPY

*FOR THE PURPOSES OF INFORMATION ONLY*

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria	FR	France	ML	Mali
AU	Australia	GA	Gabon	MR	Mauritania
BB	Barbados	GB	United Kingdom	MW	Malawi
BE	Belgium	HU	Hungary	NL	Netherlands
BG	Bulgaria	IT	Italy	NO	Norway
BJ	Benin	JP	Japan	RO	Romania
BR	Brazil	KP	Democratic People's Republic of Korea	SD	Sudan
CF	Central African Republic	KR	Republic of Korea	SE	Sweden
CG	Congo	LI	Liechtenstein	SN	Senegal
CH	Switzerland	LK	Sri Lanka	SU	Soviet Union
CM	Cameroon	LU	Luxembourg	TD	Chad
DE	Germany, Federal Republic of	MC	Monaco	TG	Togo
DK	Denmark	MG	Madagascar	US	United States of America
FI	Finland				

Aminopolycarboxylic acids and derivatives thereof

The present invention relates to certain chelating agents, in particular aminopoly(carboxylic acid or carboxylic acid derivative) compounds, and to the metal chelates thereof.

The medical use of chelating agents is well established, for example as stabilizers for pharmaceutical preparations, as antidotes for poisonous heavy metal species and as diagnostic agents for the administration of metal species (e.g. ions or atoms) for diagnostic techniques such as X-ray, magnetic resonance imaging (MRI) or ultrasound imaging or scintigraphy.

Aminopoly(carboxylic acid or carboxylic acid derivative) (hereinafter APCA) chelating agents and their metal chelates are well known and are described for example in US-A-2407645 (Bersworth), US-A-2387735 (Bersworth), EP-A-71564 (Schering), EP-A-130934 (Schering), EP-A-165728 (Nycomed AS), DE-A-2918842 (Rexolin Chemicals AB) and DE-A-3401052 (Schering).

Thus, for example, EP-A-71564 describes paramagnetic metal chelates, for which the chelating agent is nitrilotriacetic acid (NTA), N,N,N',N'-ethylenediamine-tetraacetic acid (EDTA), N-hydroxyethyl-N,N',N'-ethylenediamine-triacetic acid (HEDTA), N,N,N',N'',N''-diethylenetriamine-pentaacetic acid (DTPA) and N-hydroxyethylimino-diacetic acid, as being suitable as contrast agents for MRI, contrast being achieved by the effect of the magnetic field of the paramagnetic species (e.g. Gd(III)) with the chelating agents serving to reduce the toxicity and to assist administration of that paramagnetic species.

Amongst the particular metal chelates disclosed by EP-A-71564 was Gd DTPA, the use of which as

- 2 -

an MRI contrast agent has recently received much attention. The Gd(III) chelate of 1,4,7,10-tetraazacyclododecanetetraacetic acid (DOTA), referred to in DE-A-3401052 (Schering) and in US-A-4639365 (University of Texas), has also recently received attention in this regard.

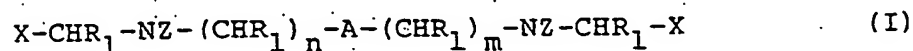
To improve stability, water solubility and selectivity, relative to the APCA chelating agents described in EP-A-71564, Schering, in EP-A-130934, have proposed the partial substitution for the N-attached carboxyalkyl groups of alkyl, alkoxyalkyl, alkoxy-carbonylalkyl or alkylaminocarbonylalkyl groups, where any amide nitrogens may themselves carry polyhydroxyalkyl groups.

However, all hitherto known APCA chelating agents and their metal chelates encounter problems of toxicity, stability or selectivity and there is thus a general and continuing need for APCA chelating agents which form metal chelates of reduced toxicity or improved stability.

We now propose certain new improved toxicity APCAs, and in particular APCAs which carry hydrophilic groups on the amine nitrogens or on the alkylene chains linking the amine nitrogens.

Viewed from one aspect, the present invention thus provides APCA chelating agents wherein at least one bridging group between amine nitrogens carries a hydrophilic moiety, preferably a hydroxyalkyl or an optionally hydroxylated alkoxy group, and metal chelates and salts thereof.

Viewed from another aspect, the present invention provides compounds of formula I



(wherein each of the groups Z is a group  $-CH(R_1)X$  or the groups Z together are a group  $-(CH(R_1))_q-A'-(CH(R_1))_r-$ , where A' is an oxygen or sulphur atom or a group  $-N-Y$ ;



- 3 -

A is a group  $\overset{|}{-N-Y}$  or  $A-(CHR_1)_m-$  represents a carbon-nitrogen bond or, when the groups Z together are a group  $-(CHR_1)_q-A'-(CHR_1)_r-$ , A may also represent an oxygen or sulphur atom;

each Y, which may be the same or different, is a

each X, which may be the same or different, is a

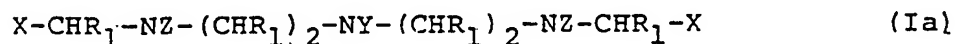
carboxyl group or a derivative thereof or a group  $R_1$ ;

each  $R_1$ , which may be the same or different, is a hydrogen atom, a hydroxyalkyl group or an optionally hydroxylated alkoxy or alkoxyalkyl group;

n,m,p,q and r are each 2,3 or 4, preferably 2;

with the provisos that at least two nitrogens carry a  $-CHR_1X$  moiety wherein X is a carboxyl group or a derivative thereof, that each  $-CHR_1X$  moiety is other than a methyl group, and that unless A' is oxygen or sulphur or A is  $N-(CHR_1)_p-N(CHR_1X)_2$  at least one  $R_1$  is other than hydrogen, preferably also that unless A' is sulphur or A is  $N-(CHR_1)_p-N(CHR_1X)_2$  at least one  $R_1$  is other than hydrogen, preferably also that where A and A' are groups  $NCHR_1X$  at least one  $CHR_1X$  group is other than a  $-CH_2X^3$  group (where  $X^3$  represents a carboxyl group or a derivative thereof or a 2-hydroxyethyl or 2,3-dihydroxypropyl group) and preferably also that each nitrogen atom carrying a  $-CHR_1X$  moiety wherein X is a carboxyl group or a derivative thereof carries at least one such moiety which is other than a  $-CH_2X$  moiety) and metal chelates and salts thereof.

Particularly preferred compounds of formula I include those of formula Ia



(wherein each group Z is a group  $-CHR_1X$  or the groups Z together are a group  $-(CHR_1)_2-A'-(CHR_1)_2-$ ;

each Y, which may be the same or different, is

a group  $-(CHR_1)_2-N(CHR_1X)_2$  or  $-CHR_1X$ ;

and A', X and R<sub>1</sub> are as hereinbefore defined) and metal chelates and salts thereof.

In the compounds of the present invention, it is particularly preferred that one or more of the -CHR<sub>1</sub>- groups in the bridges between the amine nitrogens, i.e. in the groups (CHR<sub>1</sub>)<sub>n</sub>, (CHR<sub>1</sub>)<sub>m</sub>, (CHR<sub>1</sub>)<sub>p</sub>, (CHR<sub>1</sub>)<sub>q</sub> and (CHR<sub>1</sub>)<sub>r</sub>, should carry a hydrophilic R<sub>1</sub> group. Prior art APCAs, such as DTPA or DOTA for example, possess certain hydrophobic areas which cause the metal chelates produced from such chelating agents to present relatively lipophilic and hydrophobic zones.

In the compounds of formula I, each hydrophilic R<sub>1</sub> group, which may be straight-chained or branched, preferably has a carbon atom content of from 1 to 8, especially preferably 1 to 6, carbon atoms. The R<sub>1</sub> groups may be alkoxy, polyalkoxy, polyhydroxy-alkoxy, hydroxyalkoxyalkyl or hydroxypolyalkoxyalkyl groups, but more preferably they will be monohydroxyalkyl or polyhydroxyalkyl groups. The hydrophilic R<sub>1</sub> groups serve to increase the hydrophilicity and reduce the lipophilicity of the metal chelates formed with the chelating agents of the invention and it is preferred that the compounds of formula I should contain at least 1, conveniently from 1 to 12, and preferably 2 to 8 hydrophilic R<sub>1</sub> groups and that in total the hydrophilic R<sub>1</sub> groups should contain about 6 or more hydroxy or ether oxygen atoms.

As hydrophilic R<sub>1</sub> groups, the compounds of the invention may thus include for example hydroxymethyl, 2-hydroxyethyl, 1,2-dihydroxyethyl, 3-hydroxypropyl, 2,3-dihydroxypropyl, 2,3,4-trihydroxybutyl, 1-(hydroxymethyl)-2-hydroxy-ethyl, methoxymethyl, ethoxymethyl, 2-hydroxyethoxymethyl, methoxyethoxymethyl, (2-hydroxy-ethoxy)ethyl, etc, groups.

The carboxyl derivatives which may be represented by the groups X in the compounds of formula I, may include, for example, amide groups, ester groups

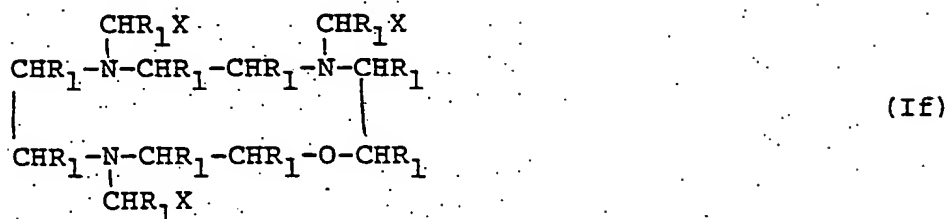
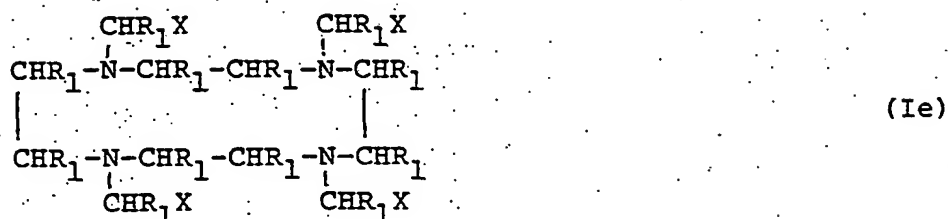
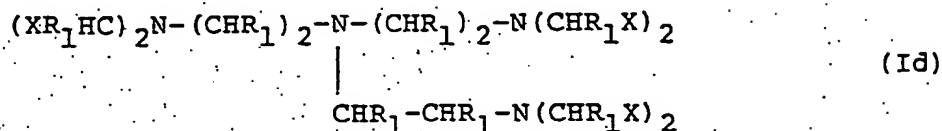
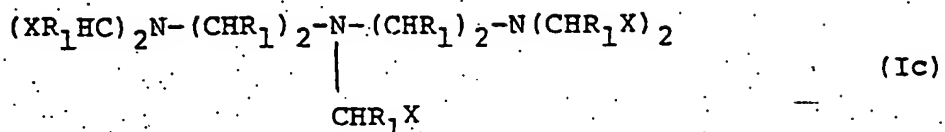
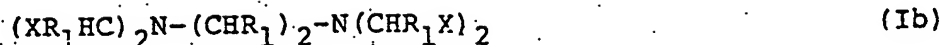
and carboxylate salt groups, for example groups of formulae  $-\text{CONR}_2\text{R}_3$  (wherein  $\text{R}_2$  is a hydrogen atom or an optionally hydroxylated alkyl, for example  $\text{C}_{1-6}$  alkyl, group and  $\text{R}_3$  is a hydrogen atom, a hydroxyl group or an optionally hydroxylated alkyl group),  $-\text{COOR}_4$  (wherein  $\text{R}_4$  is an optionally hydroxylated alkyl group), and  $-\text{COOM}$  (wherein  $\text{M}^+$  is a monovalent cation or a fraction of a polyvalent cation, for example an ammonium or substituted ammonium ion or a metal ion, for example an alkali metal or alkaline earth metal ion). Particularly preferably,  $\text{M}^+$  is a cation deriving from an organic base, for example meglumine.

It is also particularly preferred that the number of the ion-forming groups X in the compounds of formula I be chosen to equal the valency of the metal species to be chelated by the compound formula I. Thus, for example, where  $\text{Gd(III)}$  is to be chelated, the chelating agent of formula I preferably contains three ion-forming X groups, for example  $-\text{COOH}$  or  $-\text{COOM}$ . In this way, the metal chelate will be formed as a neutral species, a form preferred since the osmotic pressures in concentrated solutions of such compounds are low and since their toxicities relative to their ionic analogues are significantly reduced.

Compounds of formula I in which all the carboxyl or carboxyl derivative X groups are  $-\text{COOH}$  or  $-\text{COOM}$  groups are especially preferred since compositions containing such metal chelates can readily be sterilized, for example by autoclaving.

Included amongst the particularly preferred chelating agents of formula I are the compounds of formulae Ib, Ic, Id, Ie and If:

- 6 -



(wherein at least 2, and preferably 3 or 4, of the X groups are ion-forming groups (for example -COOH or -COOM groups), preferably wherein at least one and especially preferably at least two  $R_1$  groups are hydrophilic groups, and particularly preferably wherein at least one  $R_1$  group in each  $-(CHR_1)_2$  moiety is a hydrophilic  $R_1$  group).

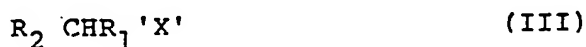
Preferred compounds of formula I include certain compounds of formulae Ib to If wherein each moiety  $-CHR_1X$  is of formula  $-CH_2X$  wherein X" is a carboxyl group or a derivative thereof.

- 7 -

Viewed from a further aspect, the invention provides a process for the preparation of the compounds of formula I, said process comprising one or more of the following steps:

- (i) reacting a corresponding amine to introduce a  $-\text{CHR}_1\text{X}$  moiety at an amine nitrogen;
- (ii) converting a carboxyl X moiety in a compound of formula I into a carboxyl derivative thereof or a carboxyl derivative X moiety in a compound of formula I into a carboxyl group; and
- (iii) converting a compound of formula I into a salt or metal chelate thereof or converting a salt or chelate of a compound of formula I into a compound of formula I.

Process step (i) may conveniently be performed by reacting a compound essentially of formula I but having at least one hydrogen atom in place of a  $-\text{CHR}_1\text{X}$  moiety and optionally having in place of X and/or  $\text{R}_1$  moieties groups convertible thereto (for example groups convertible by the removal of protecting groups), with a compound of formula III

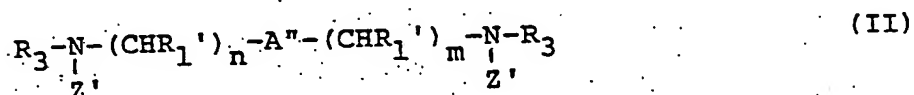


(wherein  $\text{R}_2$  is a leaving group, for example a nucleophilically displaceable group such as a halogen atom, preferably a bromine atom; and  $\text{R}_1'$  and  $\text{X}'$ , which are not both hydrogen atoms, are as defined for  $\text{R}_1$  and X or are groups convertible thereto, for example by deprotection).

As protecting groups, conventional protecting groups may be used, for example groups such as are described by T.W. Greene in "Protective Groups in Organic Synthesis", John Wiley & Sons, 1981.

For the protection of hydroxyl groups, particular mention may be made however of the utility of benzyl protecting groups which are stable over a wide pH range but are readily removed by hydrogenolysis as described by T.W. Greene. Polyhydroxyalkyl groups may for example alternatively be protected in the form of cyclic polyether groups, for example as 2,2-dimethyl-1,3-dioxo-cyclopent-4-yl groups, as such cyclic polyether groups can be opened by acid hydrolysis to leave the unprotected polyhydroxy-alkyl group.

Thus for example, introduction of a  $-\text{CHR}_1\text{X}$  moiety may be effected by reacting an amine of formula II



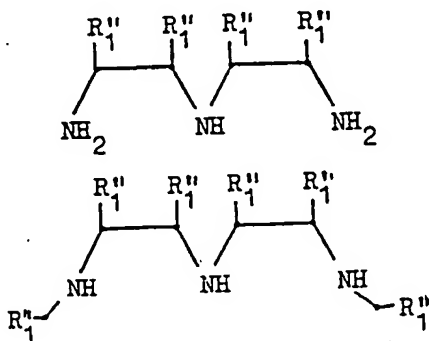
(wherein  $\text{R}_3$  is a hydrogen atom or a  $-\text{CHR}_1'\text{X}'$  group;  $\text{X}'$  and  $\text{R}_1'$  are as defined above; each group  $\text{Z}'$  is a group  $-\text{CHR}_1'\text{X}'$  or an  $\text{R}_3$  moiety or together the groups  $\text{Z}'$  are a  $-(\text{CHR}_1')_q-\text{A}''-(\text{CHR}_1')_r$  bridging group where  $\text{A}''$  is an oxygen or sulphur atom or a group  $-\text{N}-\text{Y}'$  where  $\text{Y}'$  is an  $\text{R}_3$  moiety or a group  $-(\text{CHR}_1')_p-\text{N}(\text{R}_3)_2$ ; and  $\text{A}''$  is a group  $-\text{N}-\text{Y}'$  or  $-\text{A}''-(\text{CHR}_1')_m-$  is a carbon-nitrogen bond or, where the groups  $\text{Z}'$  together form a bridging group,  $\text{A}''$  may also represent an oxygen or sulphur atom; with the provisos that at least one  $\text{R}_3$  moiety is a hydrogen atom, that at least two amine nitrogens carry a hydrogen atom or a  $-\text{CHR}_1'\text{X}'$  moiety in which  $\text{X}'$  is or is convertible to a carboxyl group or a derivative thereof, and that each  $-\text{CHR}_1'\text{X}'$  moiety is other than a methyl group) with a compound of formula III (as defined above), followed if required by converting  $\text{R}_1'$  or  $\text{X}'$  to  $\text{R}_1$  or  $\text{X}$ .

The process of step (i) is particularly preferably effected by reacting a compound of formula II (in which any hydroxyl moieties are protected) with bromoacetic acid or a derivative thereof, for example the sodium salt or an ester, followed by deprotection of the hydroxyl moieties.

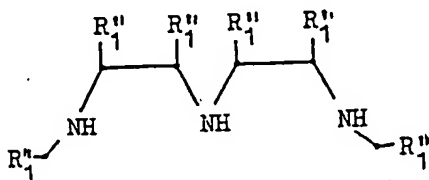
To introduce  $-\text{CHR}_1\text{X}$  groups wherein  $\text{R}_1$  is hydroxy or hydroxyalkyl, alternative compounds of formula III, such as 3-bromo-oxacyclopentan-2-one,  $\text{Hal}.\text{CH}_2\text{CH}_2\text{OH}$ ,  $\text{Hal}.\text{CH}_2\text{CHOHCH}_2\text{OH}$  or  $\text{R}_5\text{-O-CH}_2\text{-CHHal-COOH}$  (wherein Hal is a halogen atom such as a bromine atom and  $\text{R}_5$  is a protecting group) may of course be used.

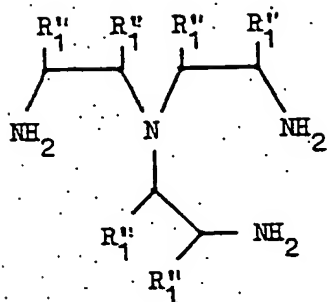
Thus for the process of step (i) the following preferred starting compounds of II may be used:

(IIa)

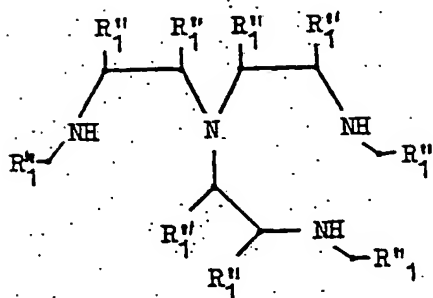


(IIb)

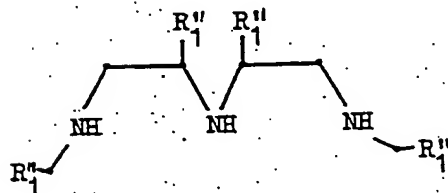




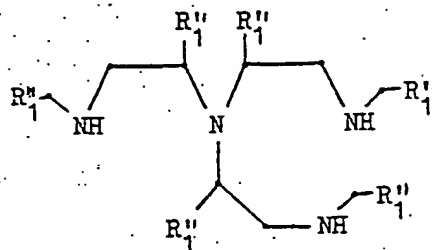
(IIc)



(IIId)

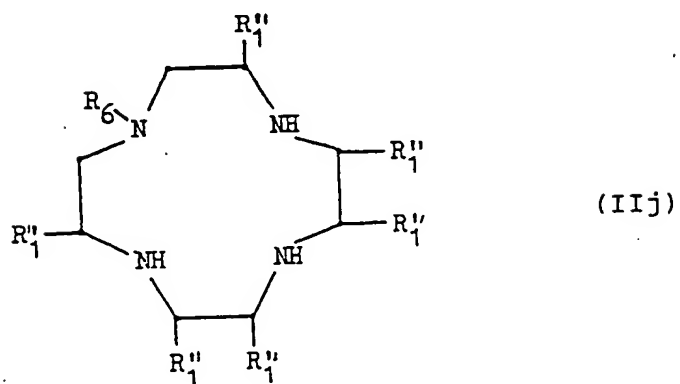
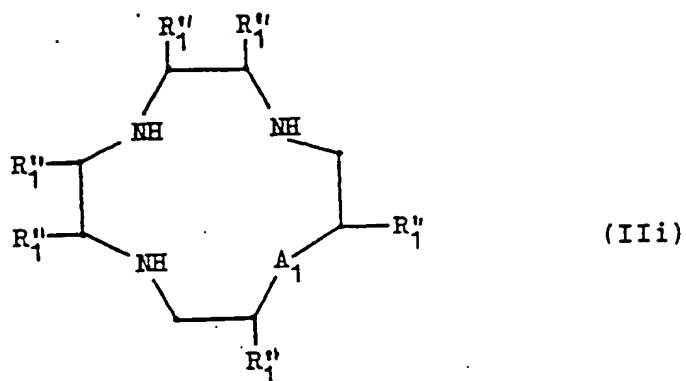
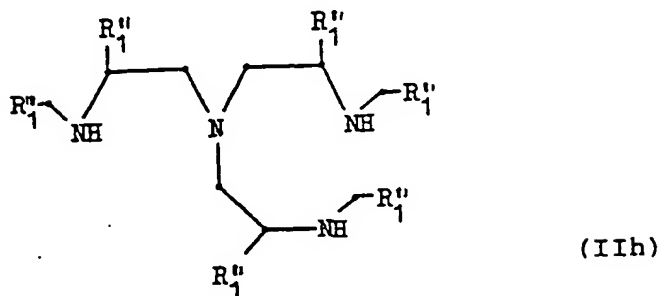
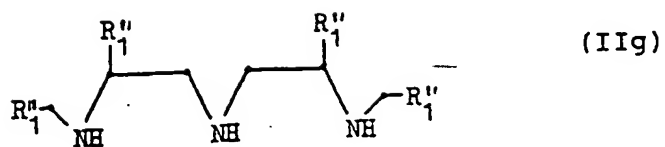


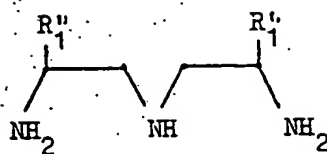
(IIe)



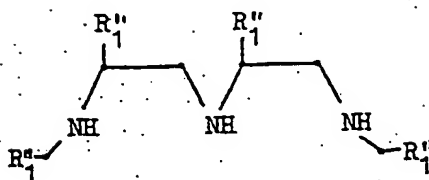
(IIIf)



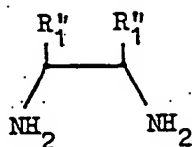




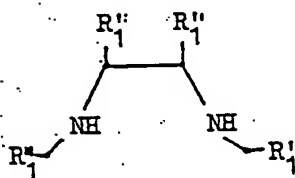
(IIk)



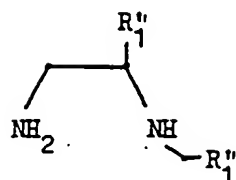
(IIl)



(IIIm)



(IIIn)

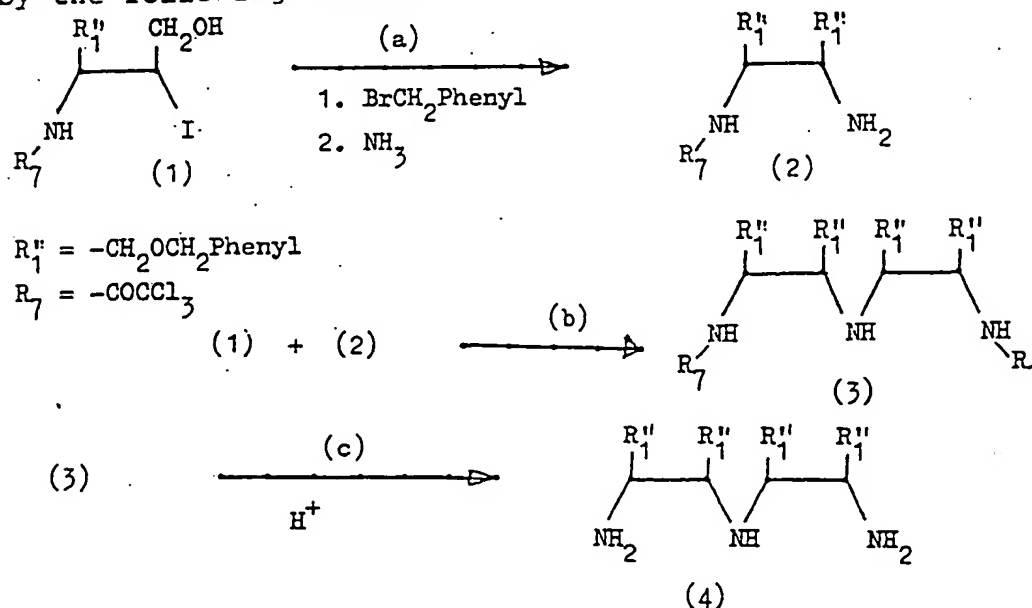


(IIIo)

(wherein each  $R_1''$  is a protected hydroxyalkyl group, for example a  $-\text{CH}_2-\text{O}-\text{CH}_2$ -Phenyl group or a 2,2-dimethyl-1,3-dioxo-cyclopent-4-yl group,  $A_1$  is a sulphur or oxygen atom or a group  $-\text{N}-\text{CHR}_1'\text{'X'}$  (for example a  $-\text{N}-\text{CH}_2\text{COOH}$  group) and  $R_6$  is a group  $-\text{CHR}_1'\text{'X'}$  or a moiety convertible thereto). In the starting compounds of formulae IIa to IIo, protected hydroxyalkyl groups attached to the alkylene chains between amine nitrogens are preferably benzyl protected groups and the nitrogen-attached protected hydroxyalkyl groups in  $-\text{CHR}_1'\text{'X'}$  moieties are preferably in the form of cyclic polyethers.

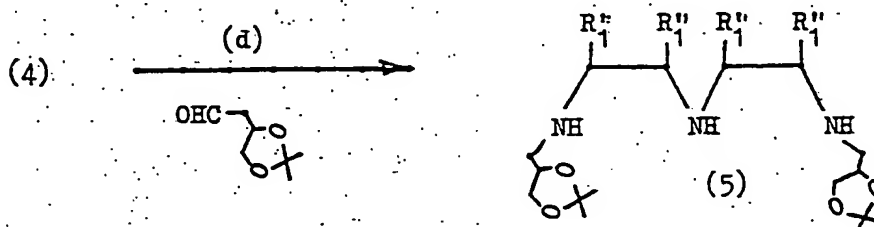
The preparation of starting compounds of formulae IIa to IIo, which compounds themselves form further aspects of the present invention, may for example be by the following procedures:

Compounds of formula IIa may be prepared by the following scheme.



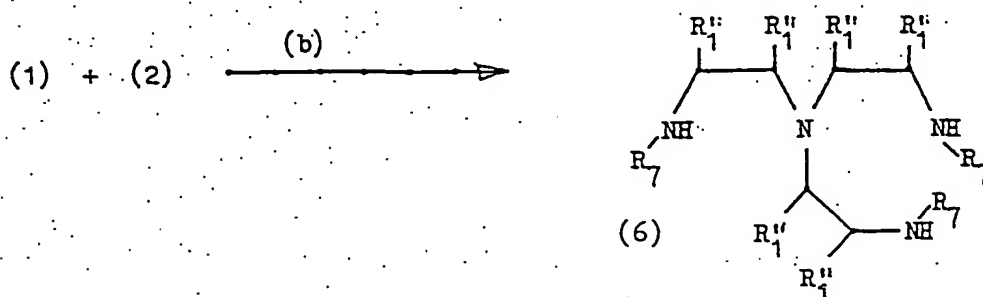
The starting compound (1) is described by A. Bongini et al. in J. Chem. Soc., Perkin Trans. 1, (1985) 935. It can be converted by benzyl protection of the remaining alcohol group and by ammonolysis to compound (2) and then condensation of compounds (1) and (2) and subsequent acid hydrolysis can yield compound (4) which is of formula IIa.

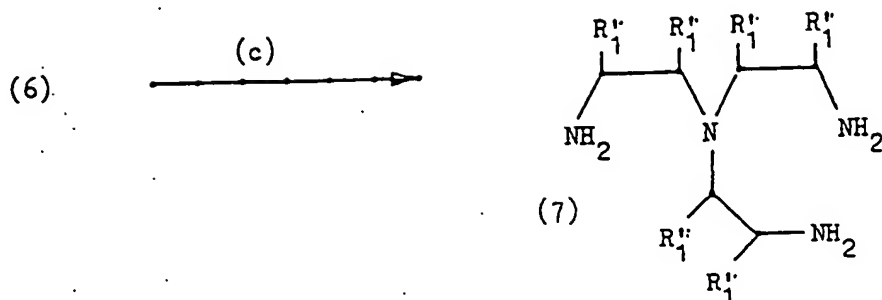
Compounds of formula IIb may be prepared from compounds of formula IIa by reductive amination, for example as described by R.F. Borch in J. Org. Chem. 34 (1969) 627, using a protected aldehyde prepared for example as described by C. Hubschwerlen in Synthesis (1986) 962. The reaction may for example follow the scheme:



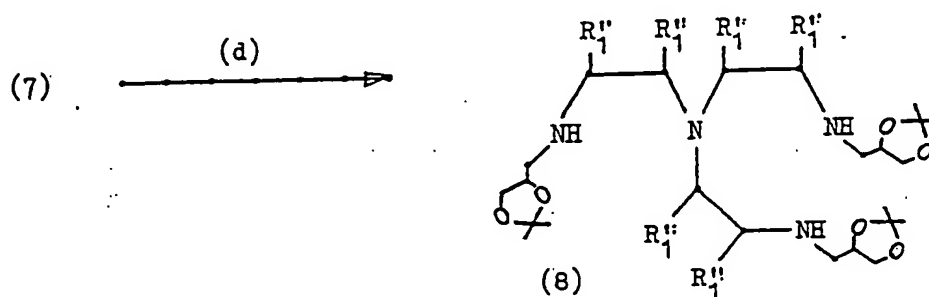
Compounds of formulae IIm and IIn may be prepared analogously to compounds of formulae IIa and IIb by omitting the condensation step (b) in the above scheme.

Compounds of formula IIc may be prepared analogously to compounds of formula IIa by condensation of compounds (1) and (2) and subsequent acid hydrolysis, for example according to the scheme:

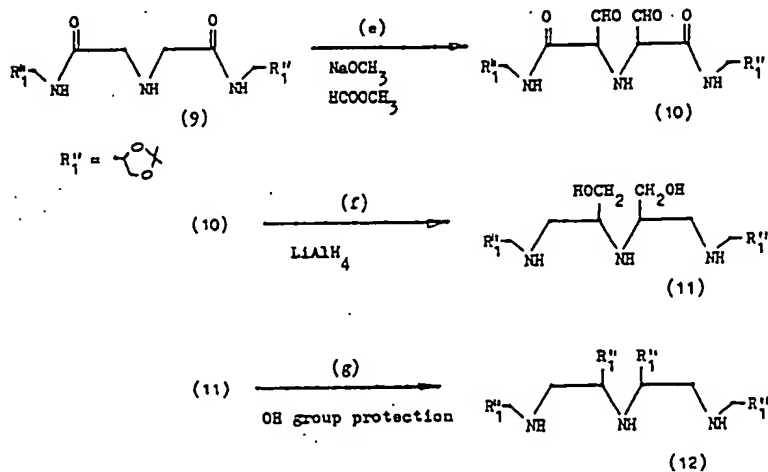




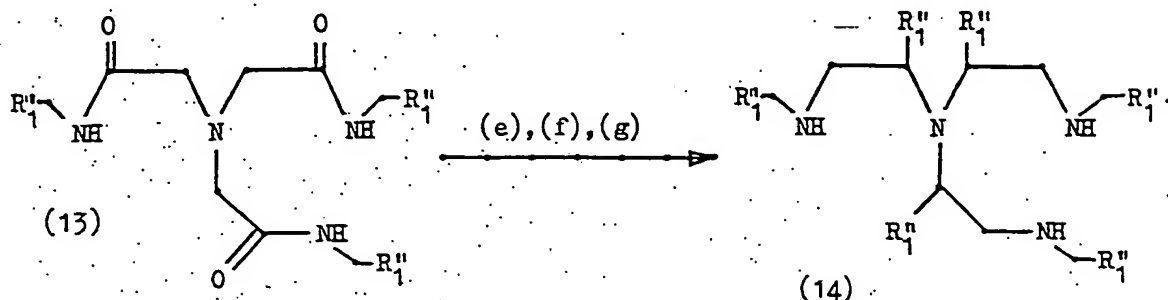
Compounds of formula IIId may be prepared analogously to compounds of formula IIb by reductive amination of compounds of formula IIc, for example following the scheme:



Compounds of formula IIe may be prepared by the following scheme:

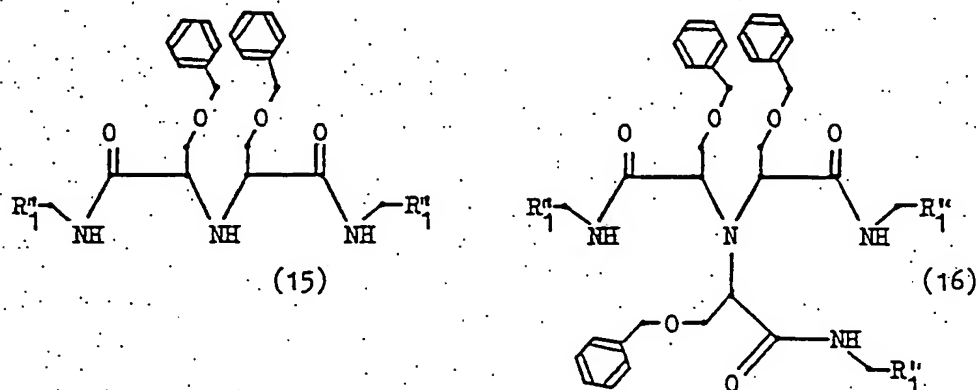


Compounds of formula II<sub>f</sub> may be prepared analogously to compounds of formula II<sub>e</sub> using a triamide starting material:



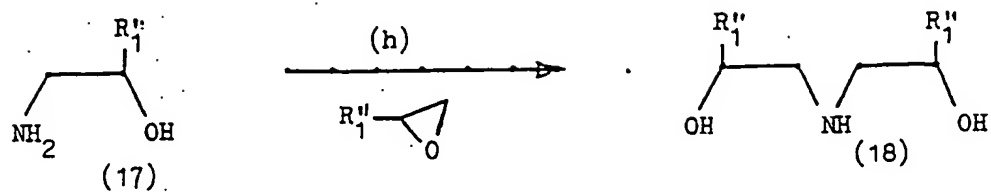
The  $\alpha$ -formylation described above may be performed using the method described in J. Med. Chem. 8 (1965) 220.

Alternatively, compounds of formulae II<sub>e</sub> and II<sub>f</sub> may be prepared by replacing the formylation step (e) by reaction with chloromethylbenzylalcohol (available from Fluka) to yield the starting compounds (15) and (16) for the reduction step (f):

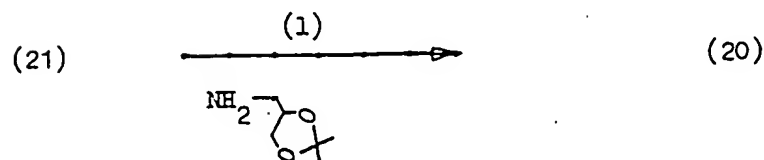
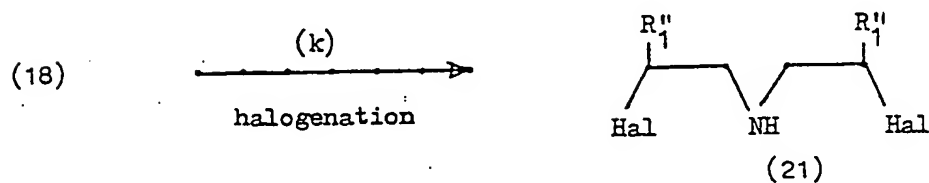
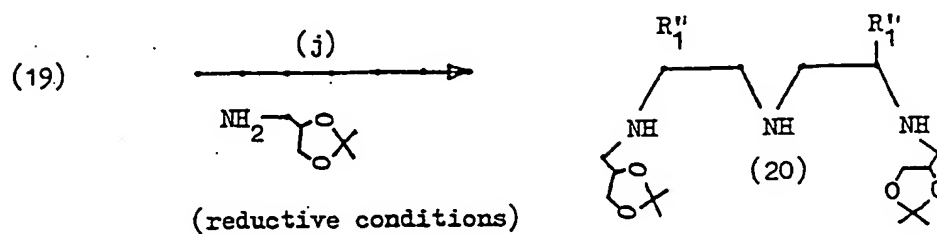
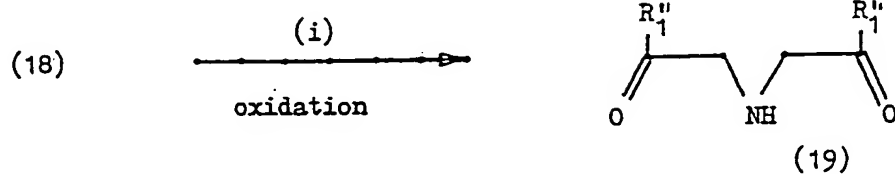


The direct insertion of benzyl-protected hydroxymethyl groups may be performed as described in Org. Syn. 52 (1972) 16.

Compounds of formula IIg may be prepared by the following scheme:

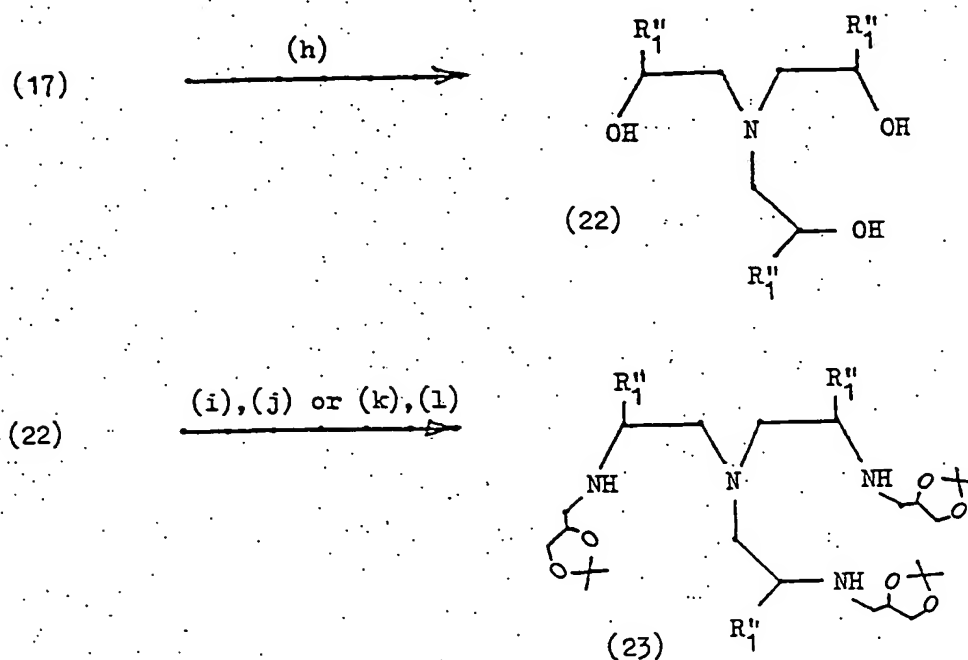


$\text{R}'' = -\text{CH}_2\text{OCH}_2\text{Phenyl}$



Compounds of formula (17) may be produced by mono-protection of aminopropandiol at the primary hydroxyl group and may be mono or di-alkylated using a glycidol ether. The mono and di-alkylation products may be separated by distillation. The mono-alkylation product, compound (18), is used in the preparation of compounds of formula IIg and the dialkylation product, compound (22) below, may be used in the preparation of compounds of formula IIh. The mono-alkylated compound (18) may be converted to compound (20), which is of formula IIg, by oxidation and subsequent reductive amination or by halogenation and subsequent amination.

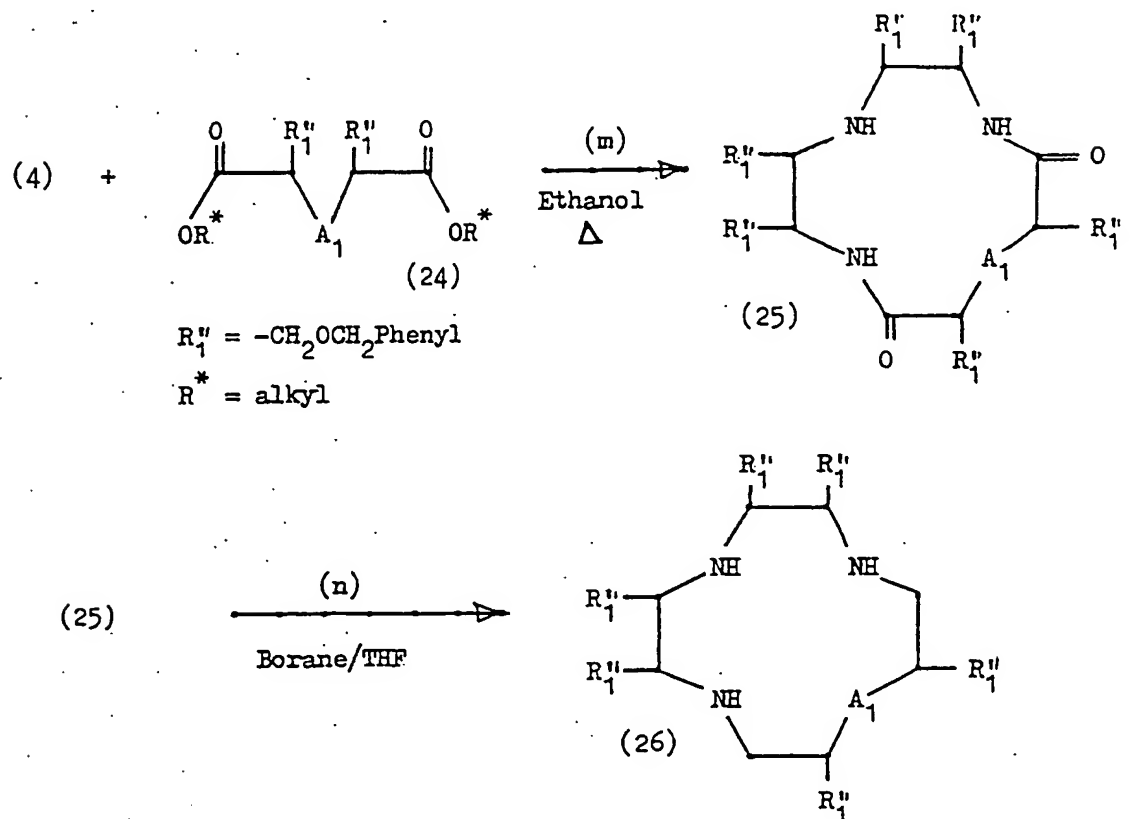
Compounds of formula IIh may be prepared analogously to the compounds of formula IIg, for example according to the scheme:



Compounds of formula IIo can be prepared analogously to compounds of formula IIg by omitting the initial mono-/di-alkylation step (h).

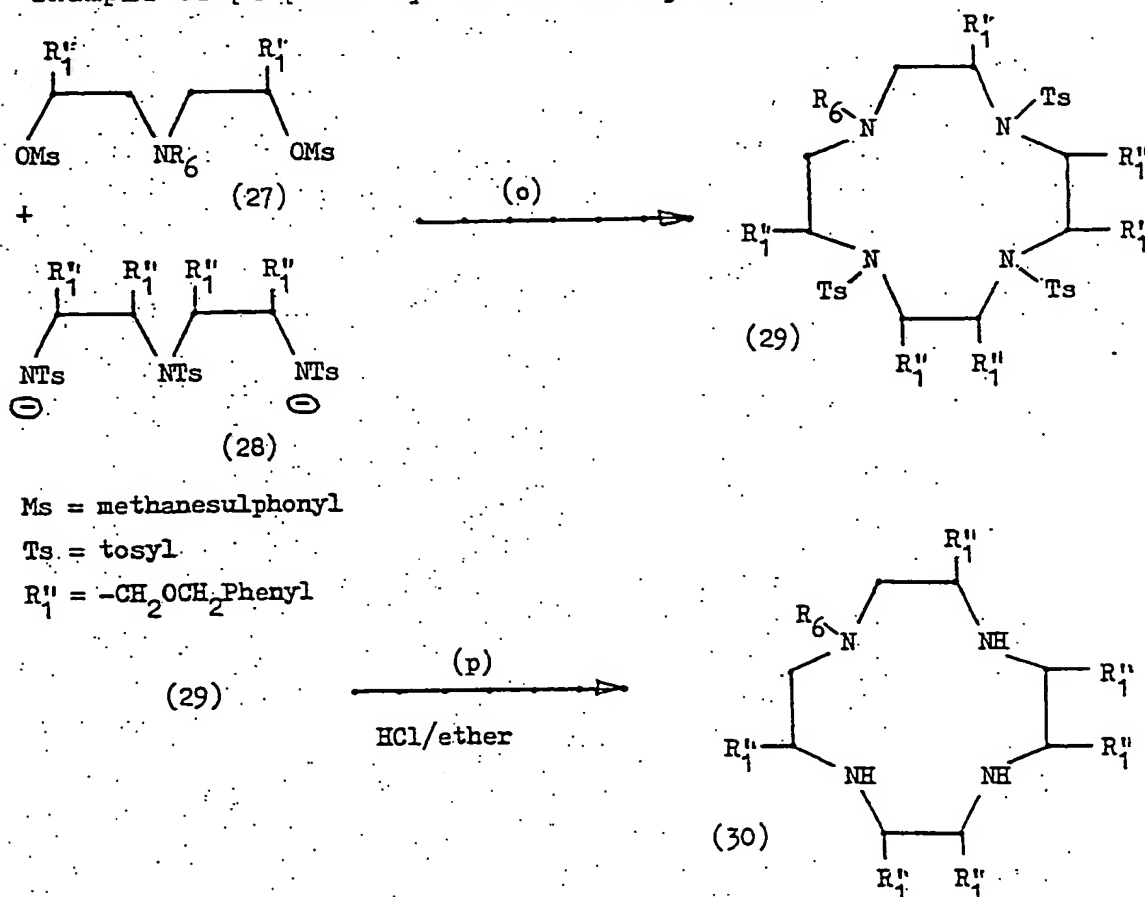


The cyclic compounds of formula Iii may be prepared by peptide condensation followed by reduction of the amide carbonyl groups, substantially as described by J. Tabushi et al. in Tetr. Lett. (1976) 4339 and (1977) 1049. The reaction may be performed according to the following scheme:



The starting compound (24), wherein  $A_1$  is an amine group, may be prepared by alkylation of an iminodiacetic acid derivative and the ether and thioether starting compounds may be prepared analogously by formylation of the corresponding starting materials, for example as described by W. Rasshofer et al. in Chem. Ber. 112 (1979) 2095. Compounds of formula Iii are particularly preferred starting materials as they may be used to form non-ionic or mono-ionic chelates with trivalent metal ions according to the selection of  $A_1$ .

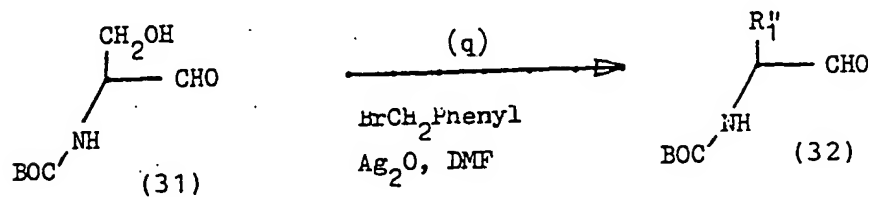
The cyclic compounds of formula IIj may be prepared by the well known routes for the preparation of cyclic polyamines. Thus, in a method analogous to that described by J.E. Richmann et al. in J. Am. Chem. Soc. 96 (1974) 2268, compounds of formula IIa may be tosylated and the resultant product may then be cyclized with a di(protected hydroxyalkyl) amine, which may itself be prepared from compound (18). Thus the compounds of formula IIj may for example be prepared by the following scheme:



Compound (27) may be prepared from compound (18) by a method analogous to that described by M. Hediger et al. in J. Chem. Soc, Chem Commun (1978) 14 and by J. Pless et al. in Chem. Abs 71 (1969) 49569x. Various detosylation methods for step (p) are known, as described for example by W. Rasshofer et al. in Liebigs Ann Chem. (1977) 1344. The group  $\text{R}_6$  may be a protecting group resistant

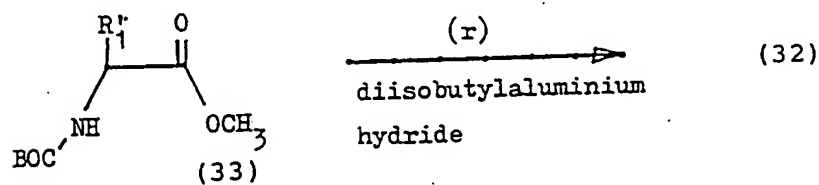
to the detosylation conditions allowing the possibility of substituting the nitrogen to which it is attached with a carboxymethyl derivative, etc.

Compounds of formula IIk may be prepared by a reaction scheme substantially as follows:

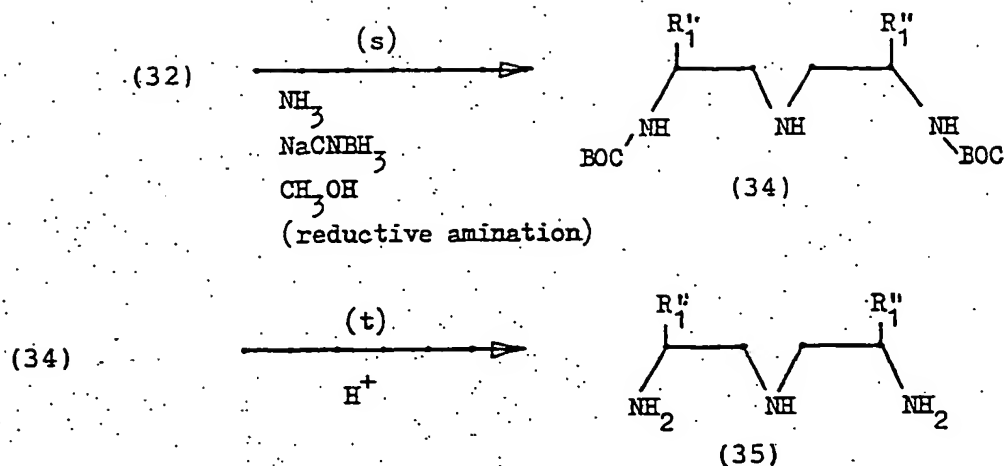


BOC = t-butyloxycarbonyl

$\text{R}_1'' = -\text{CH}_2\text{OCH}_2\text{Phenyl}$

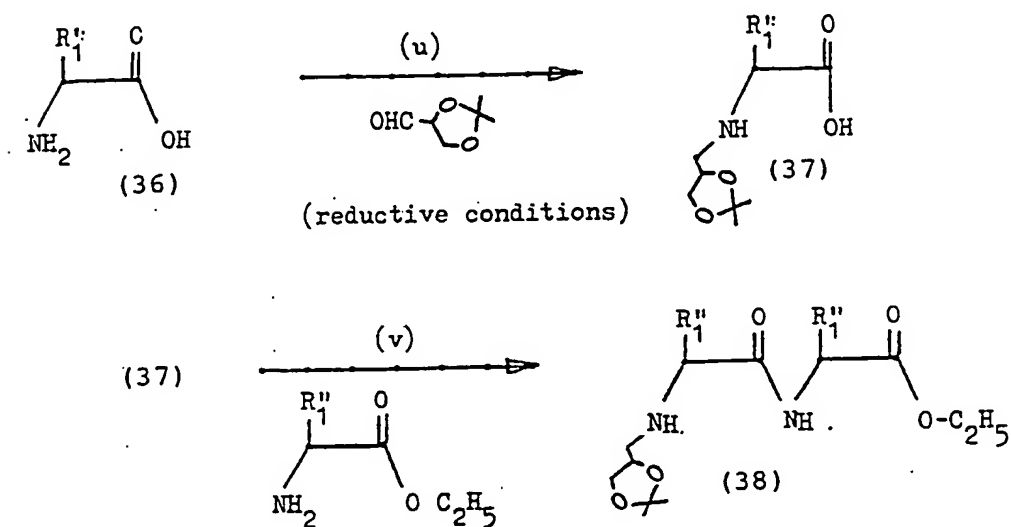


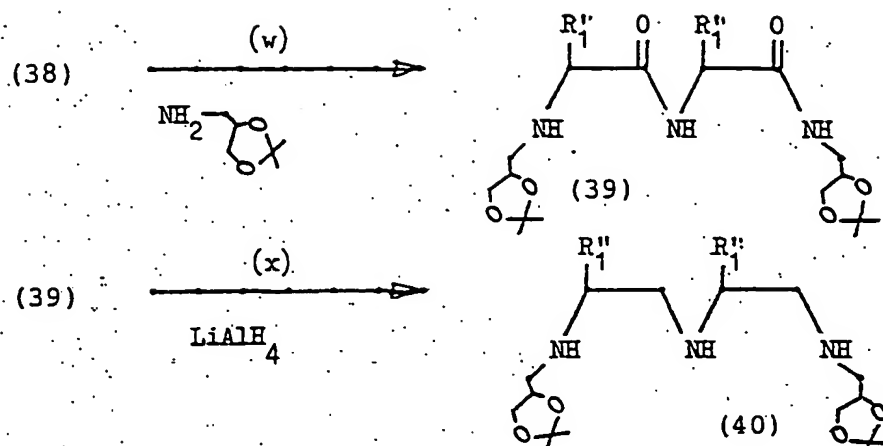
$\text{R}_1'' = -\text{CH}_2\text{OCH}_2\text{Phenyl}$



The starting compound (31) is described by Y. Ohfuné et al. in *Tetr. Lett.* (1984) 1071. Protection of the alcohol function as a benzyl ether gives compound (32) which alternatively can be obtained by reducing the commercially available serine ester, compound (33), as described in *Chem. Pharm. Bull.* 23 (1975) 3081. Reductive amination of compound (32) yields compound (34) from which the BOC (t-butyloxycarbonyl) groups may be removed by acid hydrolysis to yield compound (35) which is of formula IIk. Compounds of formula IIg may be prepared by alkylation of compounds of formula IIk, for example with 2-(2,2-dimethyl-1,3-dioxo-cyclopent-4-yl)-ethylamine. This reaction would generally be performed as a reductive amination.

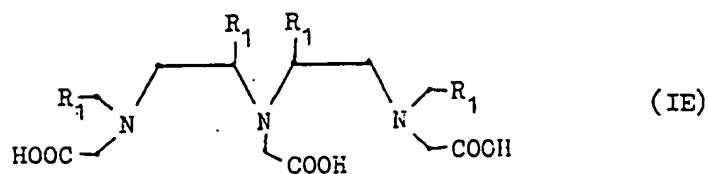
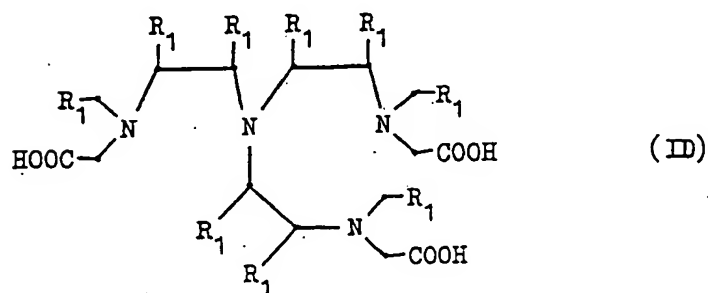
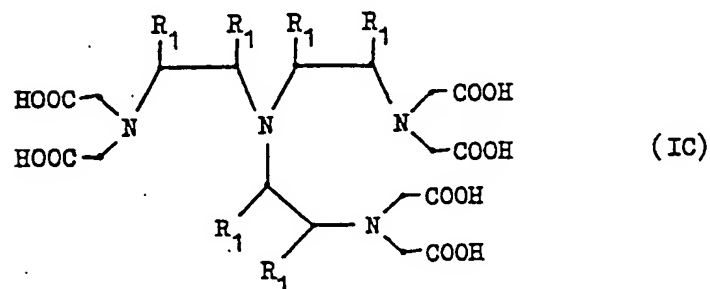
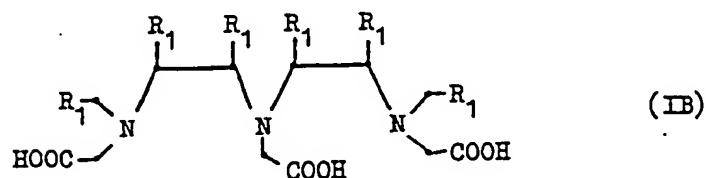
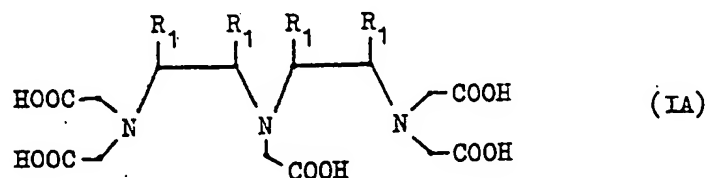
The asymmetric compounds of formula III may be prepared by peptide condensation of protected amino acids followed reduction of the amide carbonyl groups, for example using the following scheme:

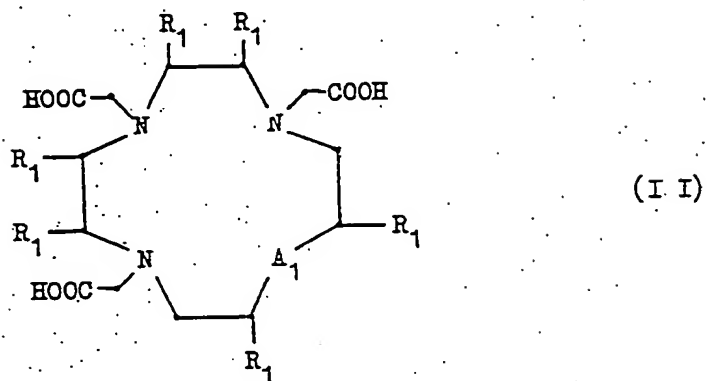
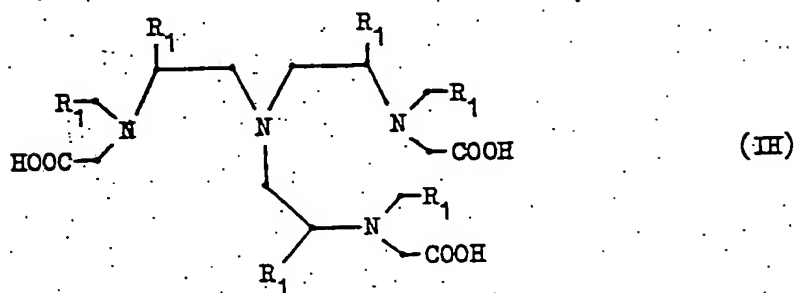
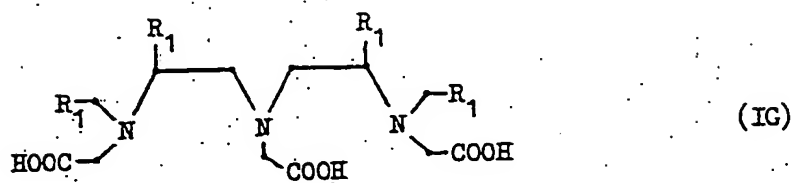
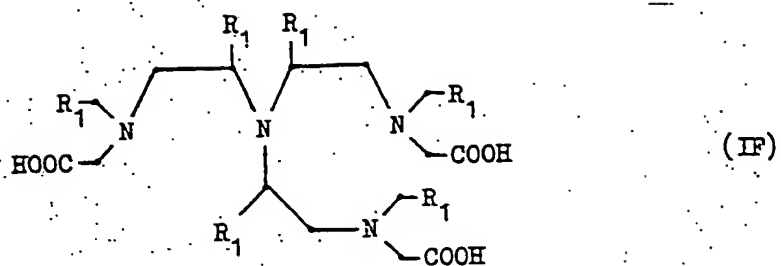




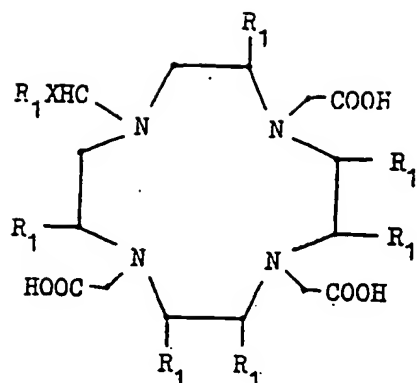
Reductive amination of O-benzyl serine with glyceraldehyde acetal gives compound (37) and coupling of compound (37) with the ethyl ester of O-benzyl protected serine gives the dipeptide compound (38) (see J. Martinez et al. Int. J. Peptide Protein Res. 12 (1978) 277). Amidation of compound (38) with a protected amino alcohol gives compound (39) which may be reduced to compound (40) using lithium aluminium hydride, as described by J. E. Nordlander et al. in J. Org. Chem. 49 (1984) 133. Compound (40) is a compound of formula III. If the amidation step (w) is omitted, reduction of compound (38) will give further asymmetric compounds of formula II.

Reaction of starting compounds of formula IIa to IIo with sodium bromoacetate and subsequent deprotection of the protected groups by acid hydrolysis or by hydrogenolysis will yield corresponding compounds of formulae IA to IO:

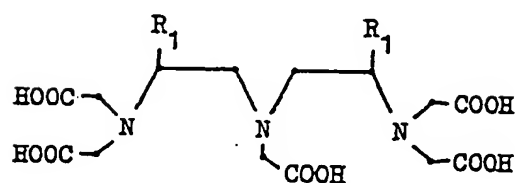




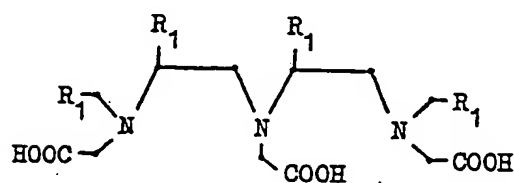




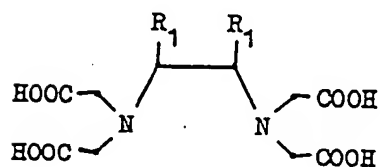
(IJ)



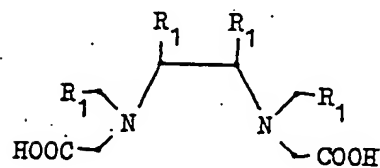
(IK)



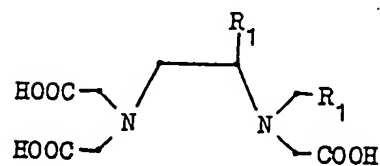
(IL)



(IM)

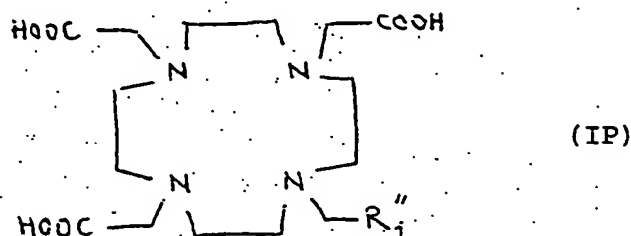


(IN)

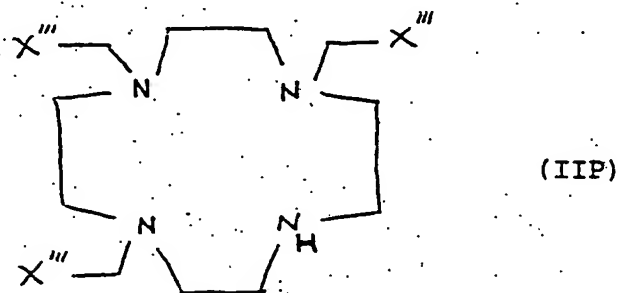


(IO)

Further compounds of formula I may be prepared in a similar fashion by the process of step (i). Thus for example a compound of formula IP



(where  $R_1''$  is as defined for  $R_1$  but does not represent a hydrogen) can be produced by reacting a compound of formula IIP



(where  $X'''$  is a protected carboxyl group, e.g. an ethoxycarbonyl group) with a compound of formula  $\text{Hal} \cdot \text{CH}_2\text{R}_1''$  (where Hal is a halogen atom) followed by deprotection of the  $X'''$  groups, e.g. by saponification. In this way for example compounds of formula IP in which  $R_1''$  is 2-hydroxyethyl or 2,3-dihydroxypropyl can be produced by reaction of a compound of formula IIP in which  $X'''$  is  $-\text{COOC}_2\text{H}_5$  with  $\text{Cl} \cdot \text{CH}_2\text{CH}_2\text{OH}$  or  $\text{ClCH}_2\text{CHOHCH}_2\text{OH}$  respectively.

The starting compound of formula IIP can be prepared by reaction of a 1,4,7,10-tetraazacyclododecane having one amine nitrogen protected by a protecting group, e.g. a benzyl group, with a compound  $\text{HalCH}_2\text{X}'''$ , e.g. ethyl bromoacetate, and subsequently removing the protecting group, e.g. using standard debenzylation conditions.

The chelating agents of the present invention are particularly suitable for use in detoxification

- 29 -

or in the formation of metal chelates, chelates which may be used for example in or as contrast agents for in vivo or in vitro magnetic resonance (MR), X-ray or ultrasound diagnostics (e.g. MR imaging and MR spectroscopy), or scintigraphy or in or as therapeutic agents for radiotherapy, and such metal chelates form a further aspect of the present invention.

For use as an MR-diagnostics contrast agent, the chelated metal species is particularly suitably a paramagnetic species, the metal conveniently being a transition metal or a lanthanide, preferably having an atomic number of 21-29, 42, 44 or 57-71. Metal chelates in which the metal species is Eu, Gd, Dy, Ho, Cr, Mn or Fe are especially preferred and  $Gd^{3+}$  and  $Mn^{2+}$  are particularly preferred. For such use, the paramagnetic metal species is conveniently non-radioactive as radioactivity is a characteristic which is neither required nor desirable for MR-diagnostics contrast agents. For use as X-ray or ultrasound contrast agents, the chelated metal species is preferably a heavy metal species, for example a non-radioactive metal with an atomic number greater than 37.

For use in scintigraphy and radiotherapy, the chelated metal species must of course be radioactive and any conventional complexable radioactive metal isotope, such as  $^{99m}Tc$  or  $^{111}In$  for example, may be used. For radiography, the chelating agent may be in the form of a metal chelate with for example  $^{67}Cu$ .

For use in detoxification of heavy metals, the chelating agent must be in salt form with a physiologically acceptable counterion, e.g. sodium, calcium, ammonium, zinc or meglumine, e.g. as the sodium salt of the chelate of the compound of formula I with zinc or calcium.

Where the metal chelate carries an overall charge, such as is the case with the prior art Gd DTPA, it will conveniently be used in the form of a salt with a physiologically acceptable counterion, for example an ammonium, substituted ammonium, alkali metal or alkaline earth metal cation or an anion deriving from an inorganic or organic acid. In this regard, meglumine salts are particularly preferred.

Viewed from a further aspect, the present invention provides a diagnostic or therapeutic agent comprising a metal chelate, whereof the chelating entity is the residue of a compound according to the present invention, together with at least one pharmaceutical or veterinary carrier or excipient, or adapted for formulation therewith or for inclusion in a pharmaceutical formulation for human or veterinary use.

Viewed from another aspect, the present invention provides a detoxification agent comprising a chelating agent according to the invention in the form of salt with a physiologically acceptable counterion, together with at least one pharmaceutical or veterinary carrier or excipient, or adapted for formulation therewith or for inclusion in a pharmaceutical formulation for human or veterinary use.

The diagnostic and therapeutic agents of the present invention may be formulated with conventional pharmaceutical or veterinary formulation aids, for example stabilizers, antioxidants, osmolality adjusting agents, buffers, pH adjusting agents, etc. and may be in a form suitable for parenteral or enteral administration, for example injection or infusion or administration directly into a body cavity having an external escape duct, for example the gastrointestinal tract, the bladder or the uterus. Thus the agent of the present invention may be in a conventional pharmaceutical administration

form such as a tablet, capsule, powder, solution, suspension, dispersion, syrup, suppository, etc; however, solutions, suspensions and dispersions in physiologically acceptable carrier media, for example water for injections, will generally be preferred.

Where the agent is formulated for parenteral administration, the carrier medium incorporating the chelate or the chelating agent salt is preferably isotonic or somewhat hypertonic.

For MR-diagnostic examination, the diagnostic agent of the present invention, if in solution, suspension or dispersion form, will generally contain the metal chelate at concentration in the range 1 micromole to 1.5 mole per litre, preferably 0.1 to 700mM. The diagnostic agent may however be supplied in a more concentrated form for dilution prior to administration. The diagnostic agent of the invention may conveniently be administered in amounts of from  $10^{-4}$  to 1 mmol of the metal species per kilogram of body weight.

For X-ray examination, the dose of the contrast agent should generally be higher and for scintigraphic examination the dose should generally be lower than for MR examination. For radiotherapy and detoxification, conventional dosages may be used.

Viewed from a further aspect, the present invention provides a method of generating enhanced images of the human or non-human animal body, which method comprises administering to said body a diagnostic agent according to the present invention and generating an X-ray, MR-diagnostics, ultrasound or scintigraphic image of at least a part thereof.

Viewed from a further aspect, the present invention provides a method of radiotherapy practised on the human or non-human animal body, which method comprises administering to said body a chelate of a radioactive metal species with a chelating agent according to the invention.

Viewed from a further aspect, the present invention provides a method of heavy metal detoxification practised on the human or non-human animal body, which method comprises administering to said body a chelating agent according to the invention in the form of a salt with a physiologically acceptable counterion.

Viewed from a yet further aspect, the present invention also provides the use of the compounds, especially the metal chelates, according to the invention for the manufacture of diagnostic or therapeutic agents for use in methods of image generation, detoxification or radiotherapy practised on the human or non-human animal body.

Viewed from a still further aspect, the present invention provides a process for the preparation of the metal chelates of the invention which process comprises admixing in a solvent a compound of formula I or a salt (e.g. the sodium salt) or chelate thereof together with an at least sparingly soluble compound of said metal, for example a chloride, oxide or carbonate.

Viewed from a yet still further aspect, the present invention provides a process for the preparation of the diagnostic or therapeutic agent of the present invention, which comprises admixing a metal chelate according to the invention, or a physiologically acceptable salt thereof, together with at least one pharmaceutical or veterinary carrier or excipient.

Viewed from a yet still further aspect, the present invention provides a process for the preparation of the detoxification agent of the invention, which comprises admixing a chelating agent according to the invention in the form of a salt with a physiologically acceptable counterion together with at least one pharmaceutical or veterinary carrier or excipient.

The present invention will now be illustrated further by the following non-limiting Examples. All ratios and percentages given herein are by weight and all temperatures are in degrees Celsius unless otherwise indicated.

Example 1

N-carboxymethyl-N,N-bis-((N'-carboxymethyl-N'-2,3-dihydroxypropyl)-2-aminoethyl)-amine

(a) To a hot, stirred solution of 276g (3.06 mol) of aminopropanediol was added dropwise 55.2g (0.29 mol) of iminodiacetic acid diethyl ester (from Tokyo Kasei) dissolved in absolute ethanol (1:1 by weight). After 3 hours at 120°C bath temperature, and evaporation of the ethanol, the excess aminopropanediol was removed by distillation at  $10^{-2}$  torr. NMR and TLC analysis showed quantitative conversion to the product diamide, with an about 10% content of aminopropanediol. The product was purified by treatment with weak acidic cation exchanger IRC (Fluka) to yield 78.5g of a yellow oil. Yield 96%.

(b) 70g (0.25 mol) of the product diamide was dissolved in 175ml of dry N,N-dimethylformamide (DMF) and 208g (1.25 mol) of 1,1-dimethoxy-ethyl-benzene and 47.5g (0.25 mol) of p-toluenesulfonic acid monohydrate was added. The solution was heated at 60°C /200 mbar in a rotary evaporator for 2.5 hours and excess dry sodium carbonate was added. Most of the solvent was evaporated and 300 ml of saturated sodium bicarbonate solution was added, after which the solution was concentrated almost to dryness. Extraction with chloroform, washing with water, drying and conventional working up yielded a yellow oil which, after 8 hours evaporation at  $10^{-3}$  mbar, gave 179g product. The product was used directly in the next step.

- 34 -

(c) To a solution of 46.8g (1.23 mol) of lithium aluminium hydride in 1.5 l of freshly distilled THF covered by nitrogen, was carefully added 37.2g (76.9 mmol) of ketal (Example 1(b)) into 200ml of tetrahydrofuran (THF). The solution was refluxed for 4 hours and the reaction product was hydrolysed at 0°C on an ice/water bath by 47ml of water in THF, by 47 ml of 15% by weight NaOH and by 141 ml of water successively. After addition of 1 litre of THF, the suspension was filtered off, washed with THF and the mother liquors were evaporated to dryness to give 25.5g (73%) of the ketal-protected triamine as a yellow oil.

(d) To the solution of 20g (43.9 mmol) of the protected triamine in 100ml of methanol in water (7:3 by volume) was added 28.3g (176 mmol) of sodium bromoacetate and the pH was kept in the alkaline range for 4 hours at 40°C. Adjustment of the pH to 3.5 and cooling on an ice/water bath gave a yellowish oil which was treated with HBr solution in acetone/water at pH 1 overnight at ambient temperature. The mixture was concentrated to half its volume, washed with chloroform and treated with a strongly cationic exchanger Dowex 50Wx4. The gel was filtered off and washed with diluted ammonia. After adjustment with formic acid to pH 3.5, the solution was concentrated to dryness under high vacuum at 60°C or by lyophilization to yield 9.3g of a white solid. Mp 51-55°C. Yield 50%.

#### Example 2

N,N,N-Tris-((N'-carboxymethyl-N'-2,3-dihydroxypropyl)-2-aminoethyl)-amine

(a) 41g (0.21 mol) of nitrilotriacetic acid was suspended in 400 ml of dry ethanol and 22.5g of concentrated sulphuric acid was added. The reaction mixture was refluxed for 4 hours while the solvent



- 35 -

was constantly evaporated. The clear solution was concentrated to dryness, dissolved in chloroform, washed with saturated sodium bicarbonate and water and dried with sodium sulphate. Work-up yielded 58.7g of a colourless oil. Yield 99.5%.

(b) To a hot, stirred solution of 164g (1.81 mol) of aminopropanediol was added dropwise 50.0g (0.18 mol) of the product nitrilotriacetic acid triethylester dissolved in absolute ethanol (1:1 by volume). After 3 hours at 120° bath temperature, and evaporation of the ethanol, the excess aminopropanediol was removed by distillation at  $10^{-2}$  torr. NMR and TLC analysis showed quantitative conversion to the product diamide, with an about 10% content of aminopropanediol. The product was purified by treatment with weak acidic cation exchanger IRC (Fluka) to yield 73.5g of a yellow oil. Yield 99.5%.

(c) 25.0g (61 mmol) of the product amide was dissolved in dry DMF and 75.9 g (0.460 mol) of 1,1-dimethoxy-ethyl-benzene and 5.96g (31 mmol) of p-toluenesulfonic acid monohydrate was added. The solution was heated at 60°C /200 mbar in a rotary evaporator for 2.5 hours and excess dry sodium carbonate was added. Most of the solvent was evaporated and 125 ml of saturated sodium bicarbonate solution was added, after which the solution was concentrated almost to dryness. Extraction with chloroform, washing with water, drying and conventional working up yielded a yellow oil which, after 8 hours evaporation at  $10^{-3}$  mbar, gave 37.7g of product. The product was used directly in the next step.

(d) To a solution of 36g (0.949 mol) of lithium aluminium hydride in 1.5 l of freshly distilled THF covered by nitrogen, was carefully added 37g

- 36 -

(52 mol) of ketal (Example 2(c)) into 200 ml THF. The solution was refluxed for 4 hours and the reaction product was hydrolysed at 0°C on an ice/water bath by 36g of water in THF, by 36g of 15% by weight NaOH and by 108g of water successively. After addition of one litre of THF, the suspension was filtered off, washed with THF and the mother liquors were evaporated to dryness to give 23.7g (68%) of the ketal-protected triamine as a yellow oil.

(e) To the solution of 23g (34 mmol) of the protected triamine in methanol-in-water (7:3 by volume) was added 18.9g (136 mmol) of sodium bromoacetate and the pH was kept in the alkaline range for 4 hours at 40°C.

Methanol was evaporated, and addition of 400ml of 20% by weight sodium chloride solution gave a yellow oil. After decantation, the oil was dissolved in acetone and water (1:1 volume), the pH was adjusted to 1 with HBr and the solution was stirred overnight. The mixture was then concentrated to half its volume, washed with chloroform and treated with a strongly cationic exchanger Dowex 50Wx4. The gel was filtered off and washed with diluted ammonia. After adjustment with formic acid to pH 3.5, the solution was concentrated to dryness under high vacuum at 60°C or by lyophilization to yield 8.3 g of a white solid. Mp 82-86 °C.

### Example 3

#### 1-Oxa-4,7,10-triazacyclododecane-N,N',N''-triacetic acid.

A solution of bromoacetic acid (2.22g (16 mmol)) and sodium hydroxide (0.64 g (16 mmol)) in 8ml of water was added dropwise to a solution of 1-

- 37 -

oxa-4,7,10-triazacyclododecane (0.80g (4.6 mmol)) in 5ml of water. (The compound 1-oxa-4,7,10-triazacyclododecane was prepared according to the procedure described by W. Raschofer, W. Wehner and F. Vogtle in Liebigs Ann. Chem., 916 (1976)).

The mixture was heated to 80°C and, while being stirred, 4M sodium hydroxide 4 ml was added dropwise. The temperature was maintained at 80°C for two hours and then brought down to ambient temperature. The pH was adjusted to 3.4 with 6N HCl. Ethanol (250ml) was added and a white crystalline precipitate was formed. The product was filtered off and washed with 50ml of ethanol. The product was further purified by treatment with a cation exchange resin (Dowex 50 Wx4). Yield : 65%.  
M.p. : 190°C (dec)

#### Example 4

#### 3,6-Bis-(carboxymethyl)-4-hydroxyethyl-3,6-diazaoctanediacid

##### (a) N-triphenylmethyl- $\alpha$ -aminobutyrolactone

$\alpha$ -Aminobutyrolactone hydrobromide (10.0 g, 54.9 mmol), N-methylmorpholine (11.2g, 110.0 mmol) and triphenylmethyl chloride (15.4 g, 55.2 mmol) were dissolved in N,N-dimethylformamide (DMF) (200 ml) (distilled from calcium hydride) and stirred at ambient temperature for 16 hours. The mixture was filtered, and water (200 ml) was added to the DMF-solution. The resulting slurry was stirred for 2 hours, the precipitate was filtered off and washed with a mixture of DMF and water (1:1, 50 ml). The solid was resuspended in water (100 ml), filtered and dried at 50°C in vacuo. N-triphenylmethyl- $\alpha$ -amino-

- 38 -

butyrolactone was isolated as a white solid. Yield 17.55 g (93.2%), mp 164.5 -165.5°C (uncorrected).

(b) 1-Carboxamido-3-hydroxy-1-(N-triphenylmethylamino)-propane

N-triphenylmethyl- $\alpha$ -aminobutyrolactone (4.0 g, 11.66 mmol) was dissolved in tetrahydrofuran (THF) (20 ml) in a 100 ml pressure vial. Methanol saturated with ammonia (20 ml) was added, and the sealed tube was heated to 70°C for 48 hours with stirring. After cooling to ambient temperature the solvent was evaporated, the residue was dissolved in ether (75 ml), n-hexane (7.5 ml) was added and the solution was kept at 4°C for 24 hours. 1-Carboxamido-3-hydroxy-1-(N-triphenylmethylamino)-propane precipitated as yellow crystals. Yield 3.0 g (71%). The structure was confirmed by  $^{13}\text{C}$ NMR and  $^1\text{H}$ NMR.

(c) 1,2-diamino-4-hydroxybutane dihydrochloride

1-Carboxamido-3-hydroxy-1-(N-triphenylmethylamino)-propane (2.5 g, 6.94 mmol) was dissolved in THF (25 ml) (distilled from lithium aluminium hydride). Lithium aluminium hydride (1.9 g, 50 mmol) was added and the mixture was refluxed overnight. Aqueous THF (50 ml, 4% water), 2N NaOH (2 ml) and aqueous THF (50 ml, 4% water) were added successively dropwise at 0°C and the mixture was stirred for 1 hour. The slurry was filtered, and the THF solution was evaporated to dryness. The resulting oil was suspended in methanol (2 ml), applied to a silica column and eluted with methanol (180 ml) and methanol: saturated aqueous ammonia 90:10 (180 ml). The last fraction was evaporated and the resulting oil (1.0 g) was dissolved in acidic methanol (25 ml containing 2 ml 12 N hydrochloric acid). After 12 hours the solvent was evaporated and 1,2-diamino-4-hydroxybu-

- 39 -

tane dihydrochloride was isolated as a white solid. Yield 0.49 g (42%), FAB MS: 105(M+1). The structure was confirmed by  $^1\text{H}$ NMR and  $^{13}\text{C}$ NMR.

(d) 3,6-Bis-(carboxymethyl)-4-hydroxyethyl-3,6-diazaoctanediacid

1,2-Diamino-4-hydroxybutane (0.35 g, 3.36 mmol) was dissolved in water (10 ml), and pH was adjusted to 9.5 with lithium hydroxide. Bromoacetic acid lithium salt (1.38 g) was added and the mixture was heated to 50°C and stirred at this temperature for 24 hours. During this period pH was kept constant (9-10) by adding a solution of lithium hydroxide (1M). The mixture was cooled to ambient temperature and applied to a Biorad AG 50W-X4 column (80 ml) and eluted with water and aqueous saturated ammonia. The title compound was isolated as a yellow solid. Yield 0.45 g (61%), mp greater than 350°C. FAB MS: 337(M+1). The structure was confirmed by  $^1\text{H}$ NMR and  $^{13}\text{C}$ NMR.

Example 5

1-Thia-4,7,10-triazacyclododecane triacetic acid

(a) Thiodiglycolic acid dimethyl ester

Thiodiglycolic acid (150.2 g, 1.0 mol) was dissolved in methanol (244 ml) and 1,2 dichloroethane (300 ml). KSF-catalyst (Aldrich) (5 g) was added and the mixture was refluxed for 14 hours. The mixture was cooled to ambient temperature and was filtered through alumina. The organic phase was separated and concentrated. Thiodiglycolic acid dimethyl ester was isolated as a yellow oil after high vacuum distillation. Yield 100 g (56%), b.p. 82-100°C (0.01 mm Hg). The structure was confirmed by  $^1\text{H}$ NMR and  $^{13}\text{C}$ NMR.

(b) 1-Thia-4,7,10-triazacyclododecane-3,11-dione

Diethylene triamine (9.3 g, 90 mmol) and thiodiglycolic acid dimethyl ester (16.2 g, 90 mmol) were dissolved in methanol (1800 ml). The mixture was refluxed for 96 hours followed by concentration to 100 ml. The precipitate was isolated by filtration, washed with methanol (10 ml) and dried. 1-Thia-4,7,10-triazacyclododecane-3,11-dione was isolated as a white solid. Yield 2.6 g (13.5%) mp 206-208°C (uncorrected). The structure was confirmed by  $^1\text{H}$ NMR and  $^{13}\text{C}$ NMR.

(c) 1-Thia-4,7,10-triazacyclododecane

1-Thia-4,7,10-triazacyclododecane-3,11-dione (8.10 g, 37.3 mmol) was suspended in dry THF (distilled from lithium aluminium hydride) (2000 ml) and refluxed under nitrogen for 8 hours with continuous addition of freshly generated diborane (approx. 0.4 mol in total). The reaction mixture was cooled to ambient temperature, 6N HCl (80 ml) was added, and the mixture was stirred for 1 hour. Sodium hydroxide (20 g, 0.5 mol) was added and the solution was evaporated to dryness. The residue was extracted with ethanol (3 x 50 ml), the extracts were concentrated and 1-thia-4,7,10-triazacyclododecane was purified by extraction (acetonitrile) and sublimation (Kugelrohr). Yield 3.73 g (53%), white solid, m.p. 51-54°C (uncorrected). The structure was confirmed by  $^1\text{H}$ NMR and  $^{13}\text{C}$ NMR.

(d) 1-Thia-4,7,10-triazacyclododecane triacetic acid

1-Thia-4,7,10-triazacyclododecane (0.378 g, 2 mmol) was dissolved in methanol (20 ml). Bromoacetic acid (1.04 g, 7.5 mmol) was dissolved in water

- 41 -

by adjusting the pH to 10 with lithium hydroxide (4M) and this aqueous solution was added to the methanol solution. The mixture was stirred at 40°C for 6 hours by repeated adjustment of pH to 9-10 with lithium hydroxide. The mixture was concentrated in vacuo, the residue was dissolved in water (5 ml) and purified on an AG 50W-X4 cation exchanger. The concentrated eluate was recrystallised from methanol/2-propanol. The title compound was isolated as a white powder. Yield 0.422 g (48%) m.p. 113-117°C. The structure was confirmed by  $^1\text{H}$ NMR and  $^{13}\text{C}$ NMR.

#### Example 6

#### $\text{N}^3, \text{N}^6, \text{N}^9$ -Tris-carboxymethyl-4,8-bis-hydroxymethyl-3,6,9-triazaundecanediacid

##### (a) N-Boc-O-benzylserine methyl ester

N-Boc-O-benzylserine-hydroxysuccinimide (16.0 g, 40.8 mmol) was dissolved in methanol (300 ml) and stirred at 50°C for 24 hours. The solvent was evaporated and the residue was taken up in ethyl acetate (120 ml), washed with aqueous saturated sodium bicarbonate (2x50 ml) and water (50 ml). The organic solution was dried with sodium sulphate and after evaporation of the solvent the N-Boc-O-benzylserine methyl ester was isolated as a colourless oil. Yield 9.9 g (83%). The structure was confirmed by  $^1\text{H}$ NMR and  $^{13}\text{C}$ NMR.

##### (b) N-Boc-O-benzylserinal

N-Boc-O-benzylserine methyl ester (9.5 g, 32 mmol) was dissolved in dry toluene (distilled from sodium) (100 ml). The solution was cooled to -70°C, and a solution of diisobutylaluminium hydride in toluene

- 42 -

(1.2M, 53 ml, 64 mmol) was added dropwise over a period of 45 minutes under vigorous stirring under nitrogen. The mixture was stirred for an additional 20 minutes and was then allowed to reach -50°C. Hydrochloric acid (2M, 150 ml) was carefully added and the resulting reaction mixture was allowed to reach -10°C. The organic layer was separated and the aqueous layer was extracted with ethylacetate (2x100 ml). The combined organic solution was dried with sodium sulphate and evaporated to dryness below 40°C (bath temperature). The N-Boc-O-benzylserinal was isolated as a colourless oil. Yield 8.6 g (96%). The structure was confirmed by  $^1\text{H}$ NMR and  $^{13}\text{C}$ NMR.

(c) N,N"-di-Boc-1,5-diamino-1,5-dibenzyloxymethyl-3-aza-pentane

N-Boc-O-benzylserinal (4.0 g, 14.3 mmol), ammonium acetate (11.4 g, 144 mmol) and sodium cyanoborohydride (0.65 g, 9.3 mmol) were stirred for 16 hours at 25°C in dry methanol (40 ml). Water (40 ml) was added and the mixture was extracted with dichloromethane (2x100 ml). The combined extracts were dried over sodium sulphate and evaporated in vacuo. N,N"-di-Boc-1,5-diamino-1,5-dibenzyloxymethyl-3-azapentane was isolated as a colourless oil. Yield 3.0 g (77%). FAB MS: 544(M+1). The structure was confirmed by  $^1\text{H}$  NMR and  $^{13}\text{C}$  NMR.

(d) 1,5-Diamino-1,5-dibenzyloxymethyl-3-azapentane

N,N"-di-Boc-1,5-diamino-1,5-dibenzyloxymethyl-3-azapentane (3.0 g, 5.5 mmol) was dissolved in dichloromethane (8.8 ml). Trifluoroacetic acid (4.4 ml) was added and the mixture was stirred for 16 hours at 25°C. The solvent and trifluoroacetic acid were removed in vacuo. The 1,5-diamino-1,5-



- 43 -

dibenzyloxymethyl-3-azapentane was isolated as a colourless oil. Yield 2.9 g (99%). The structure was confirmed by  $^1\text{H}$  NMR and  $^{13}\text{C}$  NMR.

(e)  $\text{N}^3, \text{N}^6, \text{N}^9$ -Tris-carboxymethyl-4,8-bis-hydroxymethyl-3,6,9-triazaundecanediacid

1,5-Diamino-1,5-dibenzyloxymethyl-3-azapentane (5.0 g, 14.6 mmol) in methanol (20 ml) was added to a stirred solution of bromoacetic acid (12.1 g, 87.3 mmol) and lithium hydroxide (3.7 g, 87.3 mmol) in water (20 ml). The temperature was gradually increased to 85°C during one hour and the mixture was kept stirring at 85°C for 4 hours. The reaction mixture was kept in the alkaline range during the alkylation. The mixture was then neutralised with concentrated hydrobromic acid and was loaded on a strongly cationic exchanger (AG 50WX4) and eluted with aqueous ammonia (6M) containing ammonium formate (25 mM). The crude product was dissolved in methanol (50 ml) and 10% Palladium on carbon (3 g) was added. The mixture was kept at 50°C overnight, filtered and evaporated. The title compound was isolated as a white powder. Yield 2.3 g (34%). m.p. greater than 350°C. FAB MS: 454(M+1). The structure was confirmed by  $^1\text{H}$  NMR and  $^{13}\text{C}$  NMR.

Example 7

$\text{N}^4, \text{N}^7, \text{N}^{10}$ -Triscarboxymethyl-1,13-bis-hydroxy-5,9-bis-hydroxymethyl-4,7,10-triazatridecane

(a) 2-(2-Hydroxyethyl)-1,3-dioxolane

Dowex MSA-1 (90 g, carbonate form) and 2-(2-bromoethyl)-1,3-dioxolane (18.1 g, 100 mmol) were refluxed in benzene (250 ml) for 5 hours. The resin was filtered off, washed with dichloromethane (200 ml)

- 44 -

and methanol (500 ml). The organic solvents were evaporated and the 2-(2-hydroxyethyl)-1,3-dioxolane distilled as a colourless oil. Yield 7.5 g (64%). B.p. 150°C (80 mm Hg). The structure was confirmed by  $^1\text{H}$  NMR and  $^{13}\text{C}$  NMR.

(b) 2-(2-Benzyloxyethyl)-1,3-dioxolane

2-(2-Hydroxyethyl)-1,3-dioxolane (7.0 g, 58.3 mmol) was dissolved in dried tetrahydrofuran (distilled from sodium) (200 ml). Sodium hydride (2.0 g, 66.7 mmol, 80%) was added and the mixture was stirred at ambient temperature until gas evolution was completed. Tetrabutylammonium iodide (0.22 g, 0.6 mmol) and benzyl bromide (9.7 g, 56.8 mmol) were added, and the mixture was stirred for 24 hours at ambient temperature. Diethyl ether (100 ml) was added and the mixture was washed with water (3x100 ml), dried with magnesium sulphate and 2-(2-benzyloxyethyl)-1,3-dioxolane was distilled as a colourless oil. Yield 9.3 g (75%) b.p. 75-78°C (0.02 mm Hg). The structure was confirmed by  $^1\text{H}$  NMR and  $^{13}\text{C}$  NMR.

(c) 3-Benzyloxy-propanal

A mixture of 2-(2-benzyloxyethyl)-1,3-dioxolane (2.0 g, 9.6 mmol), 35% formaldehyde in water (100 ml), tetrahydrofuran (20 ml) and sulfuric acid (0.25 g) was stirred at 50°C for 16 hours. The mixture was cooled to ambient temperature, diethyl ether (200 ml) was added and the organic phase was separated. The organic phase was washed with water (2x30 ml) and dried with magnesium sulphate and 3-benzyloxy-propanal was distilled as a colourless oil. Yield 1.2 g (76%). b.p. 150°C (40 mm Hg). The structure was confirmed by  $^1\text{H}$  NMR and  $^{13}\text{C}$  NMR.

(d) 1,13-Bisbenzyloxy-5,9-bisbenzyloxymethyl-4,7,10-triazatridecane

1,5-Diamino-1,5-dibenzyloxymethyl-3-azapentane (0.50 g, 1.5 mmol) was dissolved in methanol (20 ml). 3-Benzyl-oxypropanal (0.50 g, 3 mmol), sodium cyanoborohydride (0.11 g, 3 mmol) and 3Å molecular sieve (10 g) were added and the mixture was stirred for 16 hours at ambient temperature. The reaction mixture was filtered, water (20 ml) was added and the mixture was acidified with hydrochloric acid to pH 3. The mixture was extracted with diethyl ether (2x25 ml), the ether solution was dried with magnesium sulphate, the solution was evaporated and the mixture was chromatographed on silica. 1,13-Bisbenzyloxy-5,9-bisbenzyloxymethyl-4,7,10-triazatridecane was isolated as a white solid. Yield 0.40 g (42%), FAB MS: 640(M+1). The structure was confirmed by  $^1\text{H}$  NMR and  $^{13}\text{C}$  NMR.

(e)  $\text{N}^4, \text{N}^7, \text{N}^{10}$ -Triscarboxymethyl-1,13-bisbenzyloxy-5,9-bisbenzyloxymethyl-4,7,10-triazatridecane

1,13-Bisbenzyloxy-5,9-bisbenzyloxymethyl-4,7,10-triazatridecane (0.37 g, 0.6 mmol) was dissolved in 2-propanol (5 ml). A solution of bromoacetic acid (0.33 g, 2.4 mmol), lithium hydroxide (0.10 g, 2.4 mmol) in water (20 ml) was added. The temperature was gradually increased to reflux over five hours and kept at reflux for four hours. The reaction mixture was kept in the alkaline range during the alkylation. The solution was neutralised with concentrated hydrobromic acid and chromatographed on silica.  $\text{N}^4, \text{N}^7, \text{N}^{10}$ -Triscarboxymethyl-1,13-bisbenzyloxy-5,9-bisbenzyloxymethyl-4,7,10-triazatridecane was isolated as a white solid. Yield 0.30 g (70%) m.p. 150°C. The structure was confirmed by  $^1\text{H}$  NMR and  $^{13}\text{C}$  NMR.

(f) N<sup>4</sup>,N<sup>7</sup>,N<sup>10</sup>-Triscarboxymethyl-1,13-bishydroxy-5,9-bishydroxymethyl-4,7,10-triazatridecane

N<sup>4</sup>,N<sup>7</sup>,N<sup>10</sup>-Triscarboxymethyl-1,13-bisbenzyloxy-5,9-bisbenzyloxymethyl-4,7,10-triazatridecane (0.30 g, 0.4 mmol) was dissolved in methanol (15 ml), 10% Palladium on carbon (0.3 g) and ammonium formate (0.1 g) were added and the mixture was kept at 50°C for 16 hours. The catalyst was filtered off and washed with methanol (5 ml). The filtrate was evaporated and the title compound was isolated as a white powder. Yield 0.14 g (95%). M.p. 173-177°C. The structure was confirmed by <sup>1</sup>H NMR and <sup>13</sup>CNMR.

Example 8.

N<sup>6</sup>-Carboxymethyl-N<sup>3</sup>,N<sup>9</sup>-bis(methyl carbamylmethyl)-4,8-bishydroxymethyl-3,6,9-triazaundecane diacid

(a) N<sup>6</sup>-Carboxymethyl-N<sup>3</sup>,N<sup>9</sup>-bis(methyl carbamylmethyl)-4,8-bisbenzyloxymethyl-3,6,9-triazaundecane diacid

3,6,9-Tris-carboxymethyl-4,8-bis-hydroxymethyl-3,6,9-triazaundecanediacid (0.5 g, 0.79 mmol) (from Example 6) was dissolved in a mixture of pyridine (5 ml) and acetic anhydride (2 ml). The mixture was stirred at 65°C under a nitrogen atmosphere for 16 hours. The solvent was evaporated and the residue was dissolved in a solution of dry methylamine in chloroform (1.5M, 50 ml). The mixture was stirred for 16 hours at ambient temperature. The solvent was evaporated and the product was purified on silica. N<sup>6</sup>-Carboxymethyl-N<sup>3</sup>,N<sup>9</sup>-bis(methyl carbamylmethyl)-4,8-bis benzyloxymethyl-3,6,9-triazaundecane diacid was isolated as a colourless oil. Yield 0.30 g (57%). The structure was confirmed by <sup>1</sup>H NMR and <sup>13</sup>C NMR.

- 47 -

(b) N<sup>6</sup>-Carboxymethyl-N<sup>3</sup>,N<sup>9</sup>-bis(methyl carbamylmethyl)-4,8-bishydroxymethyl-3,6,9-triazaundecane diacid

N<sup>6</sup>-Carboxymethyl-N<sup>3</sup>,N<sup>9</sup>-bis(methyl carbamylmethyl)-4,8-bis-benzyloxymethyl-3,6,9-triazaundecane diacid (0.30 g, 0.46 mmol) dissolved in methanol (15 ml) was added to ammonium formate (0.13 g) and 10% Palladium on carbon (0.20 g). The mixture was kept at 50°C for 16 hours. The catalyst was filtered off and washed with methanol (5 ml). The filtrate was evaporated and the title compound was isolated as a white powder. Yield 209 mg, (95%). M.p. 130°C. The structure was confirmed by <sup>1</sup>H NMR and <sup>13</sup>C NMR.

Example 9

3,6,9-Tris-carboxymethyl-4-(2-hydroxyethyl)-3,6,9-triazaundecane diacid

(a) 6-Amino-2-(2-hydroxyethyl)-3-oxo-1-(tris-triphenylmethyl)-1,4-diazahexane

N-Triphenylmethyl-α-aminobutyrolactone (10 g, 29.1 mmol) (from Example 4) was dissolved in ethylenediamine (60 g, 1.0 mmol). The mixture was stirred at 50°C for 16 hours. The excess of ethylenediamine was evaporated and the oily residue was dissolved in chloroform (100 ml) and washed with water (5 x 50 ml). The organic phase was dried with sodium sulfate and evaporated. The residue was dissolved in THF (100 ml) and left overnight at 4°C. The precipitate was washed with cold THF (10 ml) and dried. 6-Amino-2-(2-hydroxyethyl)-3-oxo-1-(tris-triphenyl-methyl)-1,4-diazahexane was isolated as white crystals. Yield 8.8 g (75%) m.p. 160-161°C. The structure was confirmed by <sup>1</sup>H NMR and <sup>13</sup>C NMR.

(b) 1,5-Diamino-2-(2-hydroxyethyl)-3-azaheptane trihydrochloride

6-Amino-2-(2-hydroxyethyl)-3-oxo-1-(tris-triphenylmethyl)-1,4-diazahexane (8.8 g, 21.8 mmol) was dissolved in dry THF (distilled from lithium aluminium hydride) (10 ml). The solution was stirred under an inert atmosphere and cooled to 0°C. Lithium aluminium hydride (9.93 g, 261.9 mmol) was gradually added over 2 hours. The mixture was heated and was then refluxed for 24 hours. The reaction mixture was cooled to 0°C and aqueous THF (10 ml water in 100 ml THF) was added dropwise. 2 N Sodium hydroxide (10 ml) and water (10 ml) were added dropwise to the cooled mixture. After 2 hours the stirred suspension was filtered and the solid was washed thoroughly with THF (50 ml). The filtrate was evaporated to dryness, and the residue was eluted on silica with chloroform: methanol: ammonia in water to yield 4.5 g of a colourless oil.

The oil was dissolved in methanol (100 ml). Concentrated hydrochloric acid (10 ml) was added and the mixture was stirred at ambient temperature for 16 hours. The mixture was filtered and the filtrate was evaporated. The residue was extracted with a mixture of water (50 ml) and chloroform (50 ml). The aqueous phase was separated and evaporated. 1,5-Diamino-2-(2-hydroxyethyl)-3-azaheptane trihydrochloride was isolated as a white solid. Yield 2.8 g (50%). FAB MS: 148(M+1). The structure was confirmed by <sup>1</sup>HNMR and <sup>13</sup>CNMR.

(c) 3,6,9-Tris-carboxymethyl-4-(2-hydroxyethyl)-3,6,9-triazaundecanediacid

1,5-Diamino-2-(2-hydroxyethyl)-3-azaheptane trihydrochloride (2.8 g, 11.0 mmol) was dissolved in water

- 49 -

(10 ml). pH was adjusted to 10 with 4 M lithium hydroxide, and a solution of bromoacetic acid (9.14 g, 66 mmol) and lithium hydroxide (2.8 g, 66 mmol) in water (20 ml) was gradually added to the stirred mixture at ambient temperature. The temperature was gradually increased to 85°C during 4 hours while the pH was kept in the alkaline range (8 to 10) with aqueous lithium hydroxide. The solution was allowed to cool to ambient temperature, neutralised with concentrated hydrobromic acid, loaded on a strong cation exchanger (AG 5Wx4) and eluted with 6M aqueous ammonia with 25 mM ammonium formate. After evaporation the crude product was dissolved in water and lyophilised to yield 3.6 g (75%) of the title compound as a white solid. M.p. greater than 350°C, FAB MS: 440(M+1). The structure was confirmed by <sup>1</sup>HNMR and <sup>13</sup>CNMR.

#### Example 10

##### 3,9-Bis-(methylcarbamoylmethyl)-6-carboxymethyl-4-(2-hydroxy-ethyl)-3,6,9-triazaundecanediacid

3,6,9-Tris-carboxymethyl-4-(2-hydroxyethyl)-3,6,9-triazaundecanediacid (0.5 g, 1.13 mmol) was dissolved in a mixture of pyridine (5 ml) and acetic anhydride. The mixture was stirred under nitrogen for 16 hours at 65°C. The solvent was evaporated and the residue was dissolved in a solution of methylamine in chloroform (1.5M, 150 ml). The mixture was stirred in a pressure vial at 60°C for 24 hours. The mixture was evaporated and the residue purified on a silica column. The title compound was isolated as a white powder. Yield 0.25 g (47%). FAB MS: 462(M+1). The structure was confirmed by <sup>1</sup>HNMR and <sup>13</sup>C NMR.

Example 11Gadolinium (III) Chelate of N-carboxymethyl-N,N-bis-((N'-carboxymethyl-N'-2,3-dihydroxypropyl)-2-aminoethyl)-amine

To a solution of 42.5g (0.1 mol) of N-carboxymethyl-N,N-bis-((N'-carboxymethyl-N'-2,3-dihydroxypropyl)-2-aminoethyl)-amine (Example 1) in 300 ml water was added 18.1g (0.05 mol) of gadolinium oxide  $Gd_2O_3$  and the mixture was heated at 95°C overnight. After filtration the solution was evaporated and dried in vacuo at 50°C.

Yield : 56.6g (98%) (white powder)

M.P. : greater than 350°C

Relaxivity:  $5.0 \text{ mM}^{-1} \text{ s}^{-1}$

Example 12Gadolinium (III) Chelate of N,N,N-tris-((N'-carboxymethyl-N'-2,3-dihydroxypropyl)-2-aminoethyl)-amine

To a solution of 54.2g (0.1 mol) of N,N,N-tris-((N'-carboxymethyl-N'-2,3-dihydroxypropyl)-2-aminoethyl)amine (Example 2) in 300 ml of water was added 18.1g (0.05 mol) of gadolinium oxide  $Gd_2O_3$  and the mixture was heated at 95°C overnight. After filtration the solution was evaporated and dried in vacuo at 50°C.

Yield : 68.9g (99%) (white powder)

M.p. : greater than 350°C

Relaxivity :  $5.2 \text{ mM}^{-1} \text{ s}^{-1}$



Example 13Gadolinium (III) Chelate of 1-oxa-4,7,10-triazacyclo-dodecane-N,N',N"-triacetic acid.

A solution of 1-oxa-4,7,10-triazacyclododecane-N,N',N"-triacetic acid (0.347g (1 mmol)) (Example 3) in 10 ml water was heated to 80°C. An equimolar amount of gadolinium (III) chloride was added to the solution while the pH was continuously adjusted with 0.1 N NaOH to be within the range 5.5 - 7. The temperature was maintained at 80°C for two hours after the addition of the gadolinium chloride. The complex was isolated by evaporation to dryness.

Relaxivity :  $4.6 \text{ mM}^{-1} \text{ s}^{-1}$

Example 14Preparation of a solution containing gadolinium (III) chelate of N,N,N-tris((N'-carboxymethyl-N'-2,3-dihydroxypropyl)2-aminoethyl)-amine

6.9g of gadolinium (III) chelate of N,N,N-tris((N'-carboxymethyl-N'-2,3-dihydroxypropyl)-2-aminoethyl)-amine (the product of Example 12) was dissolved in 20 ml of distilled water. The solution was filled in a 20ml vial and autoclaved.

The solution contained 0.5 mmol Gd/ml.

Example 15Preparation of a solution containing gadolinium (III) chelate of 1-oxa-4,7,10-triazacyclododecane-N,N',N''-triacetic acid

5.01g of gadolinium (III) chelate of 1-oxa-4,7,10-triazacyclododecane-N,N',N''-triacetic acid (the product of Example 13) was dissolved in 20 ml of distilled water. The solution was filled in a 20 ml vial and autoclaved. The solution contained 0.5 mmol Gd/ml.

Example 16Preparation of a solution containing the disodium salt of the calcium chelate of 3,6-bis-(carboxymethyl)-4-hydroxyethyl-3,6-diazaoctanediacid

3,6-Bis(carboxymethyl)-4-hydroxyethyl-3,6-diazaoctanediacid (1.011 g, 3 mmol) (from Example 4) and calcium carbonate (300 mg, 3 mmol) were refluxed for 8 hours in water (15 ml). The mixture was cooled to ambient temperature, pH was adjusted to 7 by careful addition of 1N sodium hydroxide, water was added to 20 ml, the solution was filtered and filled into a 20 ml vial. The vial was autoclaved.

The solution contained 0.15 mmol calcium chelate of 3,6-bis-(carboxymethyl)-4-hydroxyethyl-3,6-diazaoctanediacid as disodium salt per ml.

The solution is for treatment of acute or chronic poisoning by heavy metals such as lead.

Example 17

Vial containing  $N^3, N^6, N^9$ -tris-carboxymethyl-4,8-bis-hydroxymethyl-3,6,9-triazaundecanediacid

A vial is filled with  $N^3, N^6, N^9$ -tris-carboxymethyl-4,8-bis-hydroxymethyl-3,6,9-triazaundecanediacid (4 mg) (Example 6) and tin (II) chloride (0.22 mg) as dry powder.

A solution of  $^{99m}\text{Tc}$  as pertechnetate in 0.9% sterile sodium chloride should be added before use. The technetium chelate with  $N^3, N^6, N^9$ -tris-carboxymethyl-4,8-bis-hydroxymethyl-3,6,9-triazaundecanediacid is for intravenous administration and is a "contrast agent" for scintigraphic examination of organs like brain and kidneys. The chelate is also useful for study of kidney function.

Example 18

Preparation of a solution of containing the disodium salt of the zinc chelate of 3,6,9-tris-carboxymethyl-4-(2-hydroxyethyl)-3,6,9-triazaundecanediacid

3,6,9-Tris-carboxymethyl-4-(2-hydroxyethyl)-3,6,9-triazaundecanediacid (878 mg, 2 mmol) (Example 9) and zinc (II) carbonate (251 mg, 2 mmol) were refluxed for 12 hours in water (15 ml). The mixture was cooled to ambient temperature, the pH was adjusted to 6 by careful addition of 1N sodium hydroxide, water was added to 20 ml, the solution was filtered and filled into a 20 ml vial. The vial was autoclaved. The solution contained 0.1 mmol of the zinc chelate as disodium salt per ml.

The solution is for treatment of acute or chronic poisoning by heavy metals such as lead and radioactive metals such as plutonium.

Example 19Preparation of a solution containing chromium (III) chelate of 3,6-bis-(carboxymethyl)-4-hydroxyethyl-3,6-diazaoctane-diacid

3,6-Bis-(carboxymethyl)-4-hydroxyethyl-3,6-diazaoctane-diacid (97 mg, 0.3 mmol) (Example 4) was dissolved in water (10 ml). Chromium (III) chloride hexahydrate (80 mg, 0.3 mmol) in water (2 ml) was added, and the mixture was stirred at 65°C for 1 hour. During the first 30 minutes N-methyl glucamine (234 mg, 1.2 mmol) was gradually added to keep pH at approximately 6. The mixture was cooled to ambient temperature and water was added to a total volume of 20 ml. The solution was filtered, filled into a 20 ml vial and autoclaved.

The concentration of chromium (III) chelate of 3,6-bis-(carboxymethyl)-4-hydroxyethyl-3,6-diazaoctane-diacid was 15 mmolar.

Example 20Bismuth (III) chelate of 1-thia-4,7,10-triazacyclododecane triacetic acid

A neutral suspension of bismuth hydroxide was prepared by neutralisation of an acidic solution of bismuth chloride (52.03 mg, 0.165 mmol) with sodium hydroxide followed by centrifugation of the precipitate and resuspension of the precipitate in water (2 ml). This suspension was added to a solution of 1-thia-4,7,10-triazacyclododecane triacetic acid (60 mg, 0.165 mmol) (Example 5) in water (2 ml) and the mixture was stirred at 80°C until complete dissolution (approximately 48 hours). The water was evaporated and the title compound isolated as a white solid. Yield 94 mg (99%) m.p. 190-220°C,  $\lambda_{\text{max}}$  = 304 nm.

Example 213,6-Bis-(carboxymethyl)-4-hydroxymethyl-3,6-diazaoctane-diacid(a) 2-Benzylloxymethyl-2-hydroxy-1-phthalimido-ethane

Freshly distilled benzyl-glycidylether (60 g, 0.365 mmol), phthalimide (49.2 g, 0.304 mmol) and potassium carbonate (3.34 g, 24 mmol) were refluxed with stirring in ethanol (500 ml) for 27 hours. TLC indicated full conversion of the starting material. Ethanol was removed in vacuo. The residual oily material was transferred to a 2 l separation funnel and distributed between ethyl acetate (800 ml) and water (500 ml). The aqueous phase was extracted with ethyl acetate (200 ml), and the combined organic phases were washed with water (300 ml), dried over magnesium sulphate, filtered and concentrated in vacuo. The residual oily material (115 g) was diluted with an equal amount (w/v) of ether (115 ml) and allowed to stand for several days. Crystals were collected on a glass sinter and air dried with suction for 1 hour; then dried under vacuum to constant weight. Yield: 81.5 g (72%). The mother liquor was diluted with ether and seeded with crystals from the first crop. Total yield: 80%. TLC indicated one spot. Structure was confirmed by NMR and IR.

(b) 2-Benzylmethyl-2-(trifluoromethylsulfonyloxy)-1-phthalimido-ethane

2-Benzylloxymethyl-2-hydroxy-1-phthalimido-ethane (20 g, 64 mmol) was dissolved in dichloromethane (180 ml), and pyridine (12.2 g, 154 mmol) was added. The mixture was cooled to -13/-15°C for 15 minutes - then trifluoromethylsulphonyloxy anhydride (triflic anhydride) (22 g, 76 mmol) was added. Without

- 56 -

further cooling, the reaction proceeded for 3 hours. TLC indicated full conversion of starting material.

The reaction mixture was transferred to a 1 l separation funnel containing dichloromethane (60 ml) and 2 M HCl (100 ml), and shaken. The aqueous phase was extracted with dichloromethane (45 ml); the combined organic phases were washed with 2 M hydrochloric acid (100 ml), saturated aqueous sodium hydrogen carbonate (100 ml) and water (100 ml), dried over magnesium sulphate, filtered and concentrated in vacuo to a pale orange solid. Yield: 28.1 g (99%). TLC indicated one spot, and the structure was confirmed by NMR and IR.

(c) 2-Benzylloxymethyl-2-azido-1-phthalimido-ethane

2-Benzylloxymethyl-2-(trifluoromethylsulfonyloxy)-1-phthalimido-ethane (8.5 g, 19.1 mmol) and sodium azide (2.5 g, 38.2 mmol) were stirred in dimethylformamide (80 ml) at 60°C for 6 hours under nitrogen. TLC in several systems was inconclusive because the R<sub>f</sub> of the product was identical to the R<sub>f</sub> of the starting material. A small sample was worked up and IR indicated full conversion to the azide. The reaction mixture was transferred to a 1 l extraction funnel and distributed between ethyl acetate (350 ml) and water (130 ml). The aqueous phase was extracted with ethyl acetate (80 ml); the combined organic phases were washed with water (2x120 ml), dried over magnesium sulphate, filtered and concentrated in vacuo to a pale brown oil. Crystallisation was initiated by adding ether (7 ml). Crystals were collected on a glass sinter, washed with cold ether and dried under vacuum for 28 hours. Yield: 4.5 g (70%). The mother liquors were concentrated, diluted with ether

and seeded with crystals from the first crop - yielding another 1 g. Total yield: 5.5 g (85%). The structure was confirmed by NMR and IR.

(d) 2-Amino-2-benzyloxymethyl-1-phthalimido-ethane

Into a stirred solution of 2-benzyloxymethyl-2-azido-1-phthalimido-ethane (500 mg, 1.5 mmol) in 4:1 pyridine/water (25 ml) was bubbled hydrogen sulphide, and the reaction was monitored by TLC. After 4 hours, the gas flow was stopped, and the reaction flask sealed. The intense green reaction mixture was stirred for another 17 hours. TLC indicated full conversion of the starting material. Glacial acetic acid was added to pH 7, and the reaction mixture was evaporated to dryness in vacuo. After evaporation from ethanol (3x25 ml), the resulting brown solid was purified by chromatography. Yield = 20%. The structure was confirmed by NMR and IR.

(e) 1,2-Diamino-1-benzyloxymethyl-ethane-dihydrochloride

1-Amino-2-benzyloxymethyl-1-phthalimido-ethane (3.0 g, 9.7 mmol) is dissolved in 50 ml dry methanol and hydrazine hydrate (968 mg, 19.3 mmol) is added. After being refluxed for 4 hours, the reaction mixture is stirred for 24 hours at ambient temperature and 50 ml 2N hydrochloric acid was added. The phthalhydrazide is removed by filtration and the 1,2-diamino-1-benzyloxymethyl-ethane-dihydrochloride is purified by recrystallisation from dry ethanol.

(f) 4-Benzyloxymethyl-3,6-bis-carboxymethyl-diaza-octanediacid

1,2-Diamino-2-benzyloxymethyl-ethane-dihydrochloride (1.2 g, 4.74 mmol) is dissolved in water (10 ml), and the pH is adjusted to 9.5 with lithium hydroxide. Bromoacetic acid lithium salt (3.49 g, 23.7 mmol) is added and the mixture is heated to 50°C and stirred at this temperature for 24 hours. During

this period the pH is kept constant (9-10) by adding a solution of lithium hydroxide (1M). The mixture is cooled to ambient temperature and applied to a Biorad AG 50W-X4 column (80 ml) and eluted with water and aqueous saturated ammonia. 4-Benzylloxymethyl-3,6-bis-carboxymethyl-diazaoctane-diacid is isolated as a yellow solid.

(g) 3,6-Bis-(carboxymethyl)-4-hydroxymethyl-3,6-diazaoctanediacid

4-Benzylloxymethyl-3,6-bis-carboxymethyl-diazaoctane-diacid (1.0 g, 2.4 mmol) is dissolved in methanol (20 ml) and 10% Palladium on carbon (5 g) is added. The mixture is kept at 50°C overnight, filtered and evaporated. The crude product is applied to a AG 50W-X4 column, washed with methanol:water (1:1) and eluted with 6M aqueous ammonia. The title compound is isolated as a white powder.

Example 22

1-(5-Hydroxy-3-oxapentyl)-1,4,7,10-tetraazacyclododecane-4,7,10-triacetic acid

(a) 1,4,7,10-Tetraazacyclododecane-4,7,10-triacetic acid tri-t-butyl ester

Sodium acetate (1.23 g, 15 mmol) was added to a stirred suspension of 1,4,7,10 tetraazacyclododecane (0.864 g, 5 mmol) (prepared in accordance with J. Am. Chem. Soc. 96 2268 (1974) and Liebigs Ann. Chem. 1340 (1977)) in N, N-dimethylacetamide (DMA) (15 ml) at ambient temperature. A solution of bromoacetic acid t-butyl ester (2.93 g, 15 mmol) in DMA (8 ml) was added dropwise to the stirred mixture, and the mixture was stirred at ambient temperature for 6 days. The solvent was evaporated and 1,4,7,10-



tetraazacyclododecane-4,7,10-triacetic acid tri-  
t-butyl ester was purified by flash chromatography  
on a silica column with chloroform:methanol 8:2  
as eluent. Yield 1.8 g (70%). White solid, m.p.  
165-175°C.

(b) 1-(5-Hydroxy-3-oxapentyl)-1,4,7,10-tetraazacyclo-  
dodecane-4,7,10-triacetic acid

1,4,7,10-Tetraazacyclododecane-4,7,10-triacetic  
acid tri-t-butyl-ester (515 mg, 1 mmol) was dissolved  
in dimethylformamide (3 ml). Sodium iodide (15 mg,  
0.1 mmol), triethylamine (233 mg, 2.5 mmol) and  
2(2-chloroethoxy)ethanol (313 mg, 3.5 mmol) were  
added to the stirred mixture at ambient temperature.  
The mixture was stirred for 18 hours at ambient  
temperature. The temperature was then raised to  
100°C and kept there for 1 hour. The solvent was  
then evaporated and the residue dissolved in a  
mixture of dichloromethane (4.5 ml) and trifluoroacetic  
acid (4.5 ml). The mixture was stirred for 2 hours  
at ambient temperature and the solvent was then  
evaporated. The residue was dissolved in water  
(8 ml), and this aqueous solution was washed with  
ether (6x4 ml). The aqueous solution was acidified  
and the title compound was isolated as a hygroscopic  
white powder after evaporation of the water. Yield  
325 mg (75%), FAB MS: 435(M+1). The structure was  
confirmed by <sup>1</sup>H NMR and <sup>13</sup>C NMR.

Example 23

1-(8-Hydroxy-3,6-dioxaoctyl)-1,4,7,10-tetraazacyclodode-  
ane-4,7,10-triacetic acid

1,4,7,10-Tetraazacyclododecane-4,7,10-triacetic  
acid tri-t-butyl ester (129 mg, 0.25 mmol) (Example  
22(a)) was dissolved in dimethylformamide (1 ml).  
Sodium iodide (7.5 mg, 0.5 mmol), triethylamine

- 60 -

(76 mg, 0.75 mmol) and 2-[2-(2-chloroethoxy)-ethoxy]-ethanol (85 mg, 0.5 mmol) were added to the stirred mixture at ambient temperature. The mixture was stirred at ambient temperature for 90 minutes, the temperature was then raised to 100°C and kept there for 1 hour. The solvent was evaporated, and the residue was dissolved in dichloromethane (2 ml) and washed with water (1 ml). Trifluoroacetic acid (2 ml) was added to the organic phase and the mixture was stirred at ambient temperature for 1 hour. The solvents were evaporated and the residue dissolved in water (4 ml). The aqueous solution was acidified, the water was evaporated and the title compound was isolated as a hygroscopic white powder. Yield 77 mg (64%). The structure was confirmed by <sup>1</sup>HNMR and <sup>13</sup>CNMR.

#### Example 24

##### 1-(2-Hydroxy-propyl)-1,4,7,10-tetraazacyclododecane-4,7,10-triacetic acid

1,4,7,10-Tetraazacyclododecane-4,7,10-triacetic acid tri-*t*-butyl ester (129 mg, 0.25 mmol) (Example 22(a)) was dissolved in dimethylformamide. Sodium iodide (7.5 mg, 0.5 mmol), triethylamine (76 mg, 0.75 mmol) and 3-chloro-2-propanol (48 mg, 0.5 mmol) were added to the stirred mixture at ambient temperature. The mixture was stirred for 4 hours at ambient temperature, the temperature was then raised to 100°C and kept there for 2 hours. The solvent was evaporated, and the residue was dissolved in dichloromethane (2 ml) and washed with water (1 ml). Trifluoroacetic acid (2 ml) was added to the organic phase and the mixture was stirred at ambient temperature for 1 hour. The solvents were evaporated and the residue dissolved in water (4 ml). The aqueous solution was washed with ether (5 x 2 ml). The aqueous solution was acidified, the water was evaporated, and the title compound was isolated as a hygroscopic

- 61 -

white powder. Yield 75 mg (74%) The structure was confirmed by  $^1\text{H}$ NMR and  $^{13}\text{C}$ NMR.

#### Example 25

##### 1-(3-Hydroxy-propyl)-1,4,7,10-tetraazacyclododecane-4,7,10-triacetic acid

1,4,7,10-Tetraazacyclododecane-4,7,10-triacetic acid tri-t-butyl ester (360 mg, 0.70 mmol) (Example 22(a)) was dissolved in dimethylformamide (2.1 ml). Sodium iodide (15 mg, 1 mmol), triethylamine (142 mg, 1.4 mmol) and 3-chloro-1-propanol (132 mg, 1.4 mmol) were added to the stirred mixture at ambient temperature. The mixture was stirred for 3 hours at ambient temperature, the temperature was then raised to 100°C and kept there for 2 hours. The solvent was evaporated and the residue was dissolved in dichloromethane (3 ml) and washed with water (1 ml). Trifluoroacetic acid (2 ml) was added to the dichloromethane solution and the mixture was stirred at ambient temperature for 1 hour. The solvents were evaporated and the residue dissolved in water (6 ml). The aqueous solution was washed with ether (6 x 3 ml). The aqueous solution was acidified, the water was evaporated, and the title compound was isolated as a hygroscopic white powder. Yield 192 mg (68%), FAB MS: 405(M+1). The structure was confirmed by  $^1\text{H}$ NMR and  $^{13}\text{C}$ NMR.

#### Example 26

##### Gd(III) chelate of 1-(5-hydroxy-3-oxa-pentyl)-1,4,7,10-tetraazacyclododecane-4,7,10-triacetic acid

1-(5-Hydroxy-3-oxapentyl)-1,4,7,10-tetraazacyclododecane-4,7,10-triacetic acid (400 mg) (Example 22) was dissolved in water (15 ml) and the pH was adjusted to 5.9. Gadolinium acetate tetrahydrate (203 mg, 0.5 mmol) was added and the mixture was stirred

- 62 -

at 60°C for 12 hours. The cooled aqueous solution was eluted through a mixed ion exchanger, the water was evaporated and the title compound was isolated as a white powder. Yield 212 mg (72%).

#### Example 27

##### Bismuth (III) chelate of $N^3, N^6, N^9$ -tris-carboxymethyl-4,8-bis-hydroxymethyl-3,6,9-triazaundecanediacid

A neutral suspension of bismuth hydroxide was prepared by neutralisation of an acidic solution of bismuth chloride (69.4 mg, 0.22 mmol) in water (3 ml) with sodium hydroxide solution followed by centrifugation of the precipitate and resuspension of the precipitate in water (2 ml). This suspension was added to a solution of  $N^3, N^6, N^9$ -tris-carboxymethyl-4,8-bis-hydroxymethyl-3,6,9-triazaundecanediacid (100 mg, 0.22 mmol) (Example 6) in water (20 ml). The pH was adjusted to 5.0 with sodium hydroxide solution, and the mixture was stirred at 95°C for 12 hours and refluxed for 7 hours. The water was evaporated and the title compound was isolated as a white solid. Yield 139 mg (95%).

#### Example 28

##### Gadolinium (III) chelate of $N^3, N^6, N^9$ -tris-carboxymethyl-4,8-bis-hydroxymethyl-3,6,9-triazaundecanediacid

A solution of gadolinium acetate tetrahydrate (89 mg, 0.22 mmol) in water (4 ml) was added to a stirred solution of  $N^3, N^6, N^9$ -tris-carboxymethyl-4,8-bis-hydroxymethyl-3,6,9-triazaundecanediacid (100 mg, 0.22 mmol) (Example 6) in water (20 ml) at ambient temperature (pH 5.7). The mixture was stirred for 90 minutes at 95°C, the solvent was evaporated

and the title compound was isolated as a white powder. Yield 128 mg (98%), FAB MS: 608(M+1).

Example 29

Gadolinium (III) chelate of 1-thia-4,7,10-triazacyclo-dodecane triacetic acid

A solution of gadolinium acetate tetrahydrate (0.13 g, 0.33 mmol) in water (3 ml) was added to a stirred solution of 1-thia-4,7,10-triazacyclododecane triacetic acid (0.12 g, 0.33 mmol) (Example 5) in water (2 ml) at ambient temperature. The pH was adjusted to 5.5 by addition of 0.1N ammonia solution and the mixture was stirred at 70°C for 22 hours (with repeated adjustment of pH). The solvent was evaporated and the title compound was isolated as a white powder. Yield 0.168 g (98%) m.p. greater than 350°C.

Example 30

Manganese (II) chelate of 1-thia-4,7,10-triazacyclo-dodecane triacetic acid

1-Thia-4,7,10-triazacyclododecane triacetic acid (0.727 g, 2 mmol) (Example 5) was dissolved in water (20 ml), and the pH was adjusted to 5.8 with 1N hydrochloric acid. Manganese carbonate (0.230 g, 2 mmol) was added and the suspension was stirred at 100°C until complete dissolution. The water was evaporated and the title compound isolated as a white hygroscopic powder. Yield 0.81 g (98%).

Example 31Fe(III) chelate of 1-(8-hydroxy-3,6-dioxaoctyl)-1,4,7,10-tetraazacyclododecane-4,7,10-triacetic acid

1-(8-Hydroxy-3,6-dioxaoctyl)-1,4,7,10-tetraazacyclododecane-4,7,10-triacetic acid (72 mg, 0.15 mmol) (Example 23) was dissolved in water (5 ml). Iron (III) chloride (24.3 mg, 0.15 mmol) was dissolved in water (5 ml), and added to the above solution. The pH was adjusted to 4 and the mixture was stirred at 100°C for 30 minutes. The water was evaporated and the title compound isolated as a brown hygroscopic powder. Yield 77 mg (97%).

Example 325,9-Bis(hydroxymethyl)-1-oxa-4,7,10-triazacyclododecane-4,7,10-triacetic acid(a) Diglycolic acid dimethyl ester

Diglycolic acid (67.05 g, 0.5 mol) was dissolved in a mixture of methanol (122 ml, 30 mol) and 1,2-dichloroethane (150 ml). KSF-catalyst (Aldrich) (2.5 g) was added and the mixture was refluxed for 15 hours. The mixture was cooled to ambient temperature, filtered through alumina, washed with aqueous saturated sodium hydrogen carbonate (100 ml) and dried over magnesium sulphate. The solvent was evaporated and diglycolic acid dimethyl ester was isolated after distillation as a colourless oil. Yield 39.5 g (49%) b.p. 65-66°C (0.055 mmHg).

(b) Diglycolic acid dichloride

Dimethylformamide (10 drops) was added to a stirred solution of diglycolic acid (13.4 g, 0.1 mmol) in thionyl chloride (50 ml) at ambient temperature. The mixture was stirred for 2 hours at 50°C and excess thionyl chloride was evaporated and the last trace of thionyl chloride was removed azeotropically with benzene. Diglycolic acid dichloride was isolated as a colourless oil. Yield 13.7 g (80%), b.p. 116°C (15 mm Hg).

(c) 5,9-Bis(benzyloxymethyl)-1-oxa-4,7,10-triazacyclododecane-3,11-dione (Alternative 1)

1,5-Diamino-1,5-dibenzyloxymethyl-3-aza-pentane (0.250 g, 0.73 mmol) (Example 6 (d)) and diglycolic acid dimethyl ester (0.118 g, 0.73 mmol) were dissolved in methanol (20 ml). The mixture was refluxed for 6 days. The solvent was evaporated and the mixture chromatographed on a silica column. 5,9-Bis(benzyloxymethyl)-1-oxa-4,7,10-triazacyclododecane-3,11-dione was isolated as a yellow oil. Yield 0.273 g (85%). FAB MS: 442(M+1).

(d) 5,9-Bis(benzyloxymethyl)-1-oxa-4,7,10-triazacyclododecane-3,11-dione (Alternative 2)

A solution of 1,5-diamino-1,5-dibenzyloxymethyl-3-aza-pentane (0.57 g, 1.7 mmol) (Example 6 (d)) and triethylamine (0.34 g, 3.4 mmol) in dichloromethane (10 ml) and a solution of diglycolic acid dichloride (0.29 g, 1.7 mmol) in dichloromethane (10 ml) were added simultaneously over 2.5 hours to vigorously stirred dichloromethane (10 ml) at 0°C. The solution was stirred at ambient temperature overnight, washed with water (2 x 30 ml), dried with sodium sulphate and evaporated. Yield 0.59 g (78%).

- 66 -

(e) 5,9-Bis-(benzyloxymethyl)-1-oxa-4,7,10-triazacyclododecane

5,9-Bis-(benzyloxymethyl)-1-oxa-4,7,10-triazacyclododecane-3,11-dione (0.250 g, 0.566 mmol) was dissolved in dry tetrahydrofuran (10 ml) cooled to 0°C. A 1 M solution of borohydride-THF complex in tetrahydrofuran (5 ml) was added under nitrogen and the mixture was stirred at ambient temperature for 14 hours. 2 M Hydrochloric acid (3 ml) was added and the mixture was concentrated in vacuo to approximately 1 ml volume. 25% Ammonia solution (2 ml) was added and the mixture was extracted four times with 4 ml of chloroform. The extracts were concentrated in vacuo and gave a yellow oil which showed an absence of carbonyl bands in IR. Yield 0.227 g (97%), FAB MS: 414(M+1).

(f) 5,9-Bis-(hydroxymethyl)-1-oxa-4,7,10-triazacyclododecane

5,9-Bis(benzyloxymethyl)-1-oxa-4,7,10-triazacyclododecane (0.24 g, 0.6 mmol) was dissolved in methanol (10 ml). Ammonium formate (0.20 g, 3 mmol) and 10% Palladium on carbon (0.40 g) were added and the mixture was stirred under a nitrogen atmosphere at 50°C for 16 hours. The catalyst was filtered off and washed with methanol (5 ml). The filtrate was evaporated and the 5,9-Bis-(hydroxymethyl)-1-oxa-4,7,10-triazacyclododecane was isolated as a colourless oil. Yield 0.12 g (86%).



- 67 -

(g) 5,9-Bis(hydroxymethyl)-1-oxa-4,7,10-triazacyclododecane-4,7,10-triacetic acid

5,9-Bis-(hydroxymethyl)-1-oxa-4,7,10-triazacyclododecane (0.12 g, 0.5 mmol) was dissolved in water (2 ml). The pH was adjusted to 10 with 4 M lithium hydroxide, and a solution of bromoacetic acid (0.21 g, 1.5 mmol) and lithium hydroxide (0.06 g, 1.5 mmol) in water (2 ml) was gradually added.

The temperature was gradually increased to 85°C during 6 hours while the pH was kept in the alkaline range with aqueous lithium hydroxide. The solution was allowed to cool to ambient temperature, neutralised with concentrated hydrobromic acid, loaded on a strong cation exchanger (AG50Wx4) and eluted with 6 M aqueous ammonia with 50 mM ammonium formate. The solution is concentrated by lyophilization and the title compound isolated. Yield: 0.09 g, (42%).

Example 33

5,9-Bis(hydroxymethyl)-1-thia-4,7,10-triazacyclododecane triacetic acid

(a) 5,9-Bis(benzyloxymethyl)-1-thia-4,7,10-triazacyclododecane-3,11-dione

1,5-Diamino-1,5-dibenzyloxymethyl-3-azapentane (0.282 g, 0.82 mmol) (Example 6(d)) and thiodiglycolic acid dimethyl ester (0.146 g, 0.82 mmol) (Example 5 (a)) were dissolved in methanol (20 ml). The mixture was refluxed for 6 days. The solvent was evaporated and 5,9-bis(benzyloxymethyl)-1-thia-4,7,10-triazacyclododecane-3,11-dione was isolated as a yellow oil after chromatography on a silica column. Yield 0.352 g (94%) FAB MS: 458 (M+1).

(b) 5,9-Bis(benzyloxymethyl)-1-thia-4,7,10-triazacyclododecane

5,9-Bis(benzyloxymethyl)-1-thia-4,7,10-triazacyclododecane-3,11-dione (0.352 g, 0.769 mmol) was dissolved in dry THF (10 ml) and cooled to 0°C. A 1 M solution of borohydride-THF complex in THF (5 ml) was added under nitrogen and the mixture was stirred for 1 hour at ambient temperature and refluxed for 0.5 hours. To the cooled solution was added 2 M hydrochloric acid (3 ml) and the resulting mixture was evaporated to dryness. Water (1 ml) and 25% ammonia solution (1 ml) was added and the mixture was extracted 3 times with 4 ml chloroform. The combined extracts were concentrated in vacuo and gave a yellow oil which showed absence of carbonyl bands in IR. Yield: 0.29 g (88%), FAB MS: 430(M+1).

(c) 5,9-Bis(hydroxymethyl)-1-thia-4,7,10-triazacyclododecane

5,9-Bis(benzyloxymethyl)-1-thia-4,7,10-triazacyclododecane (0.28 g, 0.65 mmol) was dissolved in methanol (10 ml). Ammonium formate (0.39 g) and 10% Palladium on carbon (1.3 g) were added under nitrogen and the mixture was stirred at 50°C for 16 hours. The catalyst was filtered off and washed with methanol (5 ml). The filtrate was evaporated and 5,9-bis(hydroxymethyl)-1-thia-4,7,10-triazacyclododecane was isolated as a white solid. Mp. 210-220°C (subl.). Yield: 0.099 g (61%).

(d) 5,9-Bis(hydroxymethyl)-1-thia-4,7,10-triazacyclododecane triacetic acid

5,9-Bis(hydroxymethyl)-1-thia-4,7,10-triazacyclododecane (0.090 g, 0.361 mmol) is dissolved in water (2 ml). The pH is adjusted to 10 with 4M lithium hydroxide,

- 69 -

and a solution of bromoacetic acid (0.170 g, 1.226 mmol) in water (2 ml) is gradually added. The temperature is gradually increased to 85°C during 4 hours while the pH is kept in the alkaline range (8 to 10) with aqueous lithium hydroxide. The solution is allowed to cool to ambient temperature, neutralised with concentrated hydrobromic acid, loaded on a strong cation exchanger (AG 50Wx4) and eluted with 6M aqueous ammonia with 25mM ammonium formate. The solution is evaporated and the title compound is isolated.

#### Example 34

Preparation of a solution containing the calcium salt of the gadolinium (III) chelate of  $N^3, N^6, N^9$ -triscarboxymethyl-4,8-bis-hydroxymethyl-3,6,9-triazaundecanediacid

Gadolinium (III) chelate of  $N^3, N^6, N^9$ -triscarboxymethyl-4,8-bis-hydroxymethyl-3,6,9-triazaundecanediacid (607 mg, 1 mmol) (Example 28) and calcium hydroxide (74 mg, 1 mmol) were suspended in water (8 ml). The mixture was refluxed for 5 hours, the pH was adjusted to 7.0 and water (to 10 ml) was added. The solution was filtered and filled into a 10 ml vial. The vial was autoclaved. The solution contained 0.1 mmol Gd per ml.

#### Example 35

Preparation of a solution containing the di-lysine salt of the gadolinium (III) chelate of  $N^3, N^6, N^9$ -triscarboxymethyl-4,8-bis-hydroxymethyl-3,6,9-triazaundecanediacid

Gadolinium (III) chelate of  $N^3, N^6, N^9$ -triscarboxymethyl-4,8-bis-hydroxymethyl-3,6,9-triazaundecanediacid (3.04 g, 5 mmol) (Example 28) and lysine (2.92 g, 10 mmol) were suspended in water (8 ml). The mixture

- 70 -

was refluxed for 4 hours, the pH was adjusted to 7.0 and water (to 10 ml) was added. The solution was filtered and filled into a 10 ml vial. The vial was autoclaved. The solution contained 0.5 mmol Gd per ml.

### Example 36

N<sup>6</sup>-Carboxymethyl-N<sup>3</sup>,N<sup>9</sup>-bis(2,3-dihydroxypropyl-carbamyl-methyl)-4,8-bis(hydroxymethyl)-3,6,9-triazaundecane diacid

(a) N<sup>6</sup>-Carboxymethyl-N<sup>3</sup>,N<sup>9</sup>-bis(2,3-dihydroxypropyl-carbamylmethyl)-4,8-bis(benzyloxymethyl)-3,6,9-triazaundecane diacid

3,6,9-Tris-carboxymethyl-4,8-bis(benzyloxymethyl)-3,6,9-triazaundecanediacid (0.5 g, 0.79 mmol) (Example 6) was dissolved in a mixture of pyridine (5 ml) and acetic anhydride (2 ml). The mixture was stirred at 65°C under a nitrogen atmosphere for 16 hours. The solvent was evaporated and the residue was dissolved in dimethylformamide (5 ml). A solution of 1-amino-2,3-propanediol (0.7 g, 7.8 mmol) in dimethylformamide (2 ml) was added. The solution was stirred for 16 hours and the solvent and excess reagent were evaporated. N<sup>6</sup>-Carboxymethyl-N<sup>3</sup>,N<sup>9</sup>-bis(2,3-dihydroxypropyl-carbamylmethyl)-4,8-bis(benzyloxymethyl)-3,6,9-triazaundecane diacid was isolated as a colourless oil. Yield 0.32 g (52%). The structure was confirmed by <sup>1</sup>H NMR and <sup>13</sup>C NMR.

(b) N<sup>6</sup>-Carboxymethyl-N<sup>3</sup>,N<sup>9</sup>-bis(2,3-dihydroxypropyl-carbamylmethyl)-4,8-bis(hydroxymethyl)-3,6,9-triazaundecane diacid

N<sup>6</sup>-Carboxymethyl-N<sup>3</sup>,N<sup>9</sup>-bis(2,3-dihydroxypropyl-carbamylmethyl)-4,8-bis(benzyloxymethyl)-3,6,9-triazaundecane diacid (0.32 g, 0.41 mmol) was dissolved.

- 71 -

in methanol (15 ml). Ammonium formate (0.13 g) and 10% Palladium on carbon (0.20 g) were added and the mixture was stirred at 50°C for 16 hours. The catalyst was filtered off and washed with methanol (5 ml). The filtrate was evaporated and the title compound was isolated as a colourless oil. Yield 0.23 g (95%). The structure was confirmed by  $^1\text{H}$  NMR and  $^{13}\text{C}$  NMR.

### Example 37

Solution containing gadolinium (III) chelate of 1-oxa-4,7,10-triazacyclododecane-N,N',N''-triacetic acid for parenteral administration

Gadolinium (III) chelate of 1-oxa-4,7,10-triazacyclododecane-N,N',N''-triacetic acid

(Example 13)	0.50 g
Saccharin sodium	1.0 g
Ethanol	10.0 g
Orange essence	0.3 g
Apricot essence	0.7 g
Water	<u>ad</u> 1000 ml

Gadolinium (III) chelate of 1-oxa-4,7,10-triazacyclododecane-N,N',N''-triacetic acid was dissolved in water (500 ml). The essences were dissolved in ethanol and slowly added to the aqueous solution. Water was added to bring the volume to 1000 ml and the solution was filled into a 1000 ml vial. The solution contained 1 mmol gadolinium per litre.

### Example 38

2-(2-Hydroxyethyl)-1,4,7,11-tetrakis-carboxy-methyl-1,4,7,11-tetraazacyclododecane

(a) 2-(2-Hydroxyethyl)-3-oxo-1,7-bis(triphenylmethyl)-1,4,7-triazaheptane

6-Amino-2-(2-hydroxyethyl)-3-oxo-1-(triphenylmethyl)-1,4-diazaheptane (15.0 g, 37.2 mmol) (Example 9(a)) was dissolved in 150 ml DMF and triphenylmethyl chloride (10.35 g, 37.2 mmol) and methylmorpholine (3.75 g, 37.2 mmol) was added. After being stirred for 24 hours at ambient temperature, the reaction mixture was filtered and poured into 200 ml of ice water. After 1 hour stirring, filtration and thoroughly washing the precipitate with water, the solid was dried in vacuo at 50°C to yield 22 g of crude product. The product was dissolved in chloroform/toluene/methanol (80/16/14) and purified on silica to yield 6.6 g (27%) of 2-(2-hydroxyethyl)-3-oxo-1,7-bis(triphenylmethyl)-1,4,7-triazaheptane as white crystals, m.p. 71-72°C, FAB MS: 646(M+1). The structure was confirmed by <sup>13</sup>C NMR.

(b) 2-(2-Benzylloxyethyl)-3-oxo-1,7-bis(triphenylmethyl)-1,4,7-triazaheptane

2-(2-Hydroxyethyl)-3-oxo-1,7-bis(triphenylmethyl)-1,4,7-triazaheptane (10 mmol) is reacted as described by S. Czernecki et al. Tetr. Lett. (1976) 3535 with 80% sodium hydride (11 mmol) and benzylbromide (10 mmol) catalysed by tetrabutyl ammonium iodide (1 mmol) for 24 hours at ambient temperature (in 100 ml dry THF). 2-(2-Benzylloxyethyl)-3-oxo-1,7-bis(triphenylmethyl)-1,4,7-triazaheptane is purified by chromatography on silica.

(c) 1,5-Diamino-1-(2-benzylloxy-ethyl)-3-aza-pentane hydrochloride

2-(2-Benzylloxyethyl)-3-oxo-1,7-bis(triphenylmethyl)-1,4,7-triazaheptane is reacted in a manner analogous

- 73 -

to the reaction of step (c) of Example 9 up to the point where the purified product is taken up in methanol and conc. hydrochloric acid is added. From this point the method described by M. Bessodes *et al.*, Tetr. Lett., (1986) 579 to remove the triphenylmethyl protecting groups is employed: the product is taken up in a mixture of formic acid and diethylether and refluxed for several hours. After evaporation to dryness the product is dissolved in water, the pH is adjusted to 10 and the product is extracted with chloroform. The organic phase is evaporated and the product is taken up in dry ethanol. Dry hydrogen chloride gas is bubbled through the stirred solution cooled with an ice/water bath, and 1,5-diamino-1-(2-benzyloxy-ethyl)-3-azapentane hydrochloride is collected by filtration.

(d) 2-(2-Benzyloxyethyl)-tris-1,4,7-(4-methyl-benzenesulphonyl)-1,4,7-triazaheptane

1,5-Diamino-1-(2-benzyloxy-ethyl)-3-aza-pentane hydrochloride (10 mmol) is dissolved in 20 ml of water and the pH is adjusted to 10.5. Tosyl chloride (30 mmol) dissolved in ether (15 ml) is added dropwise while the reaction mixture is vigorously stirred at ambient temperature for 24 hours. The ether phase is gradually evaporated to a minimum and the white solid is collected and purified on silica to yield 2-(2-benzyloxyethyl)-tris-1,4,7-(4-methyl-benzenesulphonyl)-1,4,7-triazaheptane.

(e) 2-(2-Hydroxyethyl)-1,4,7,11-tetraazacyclododecane

2-(2-Benzyloxyethyl)-tris-1,4,7-(4-methyl-benzene-sulphonyl)-1,4,7-triazaheptane (10 mmol) is dissolved in 20 ml dry ethanol and a solution of sodium ethanoate (20 mmol) in 20 ml ethanol is added. The reaction mixture

is refluxed for 1 hour, the solvent is evaporated and the residue is taken up in dry dimethylformamide (100 ml); during 15 minutes this solution is added to a solution of diethanolamine tritosylate (10 mmol) (prepared according to the method of Richman *et al.* J. Am. Chem. Soc. 96 (1974) 2268) in 50 ml dry DMF.

After 2 hours stirring at 110°C the solution is poured into 150 ml ice water and the solid is collected by filtration and dried in vacuo. The solid is stirred in conc. sulfuric acid and water at 110°C for 45 hours. The suspension is diluted with 6N hydrochloric acid and THF and after cooling the crystallisate is collected and 2-(2-hydroxyethyl)-1,4,7,11-tetraazacyclododecane is purified by chromatography.

(f) 2-(2-Hydroxyethyl)-1,4,7,11-tetrakis-carboxymethyl-1,4,7,11-tetraazacyclododecane

2-(2-Hydroxyethyl)-1,4,7,11-tetraazacyclododecane (10 mmol) is dissolved in 50 ml water and the pH is adjusted to 10 using 4 M lithium hydroxide solution. Bromoacetic acid lithium salt is added at 60°C and the pH is kept alkaline while the temperature is gradually increased to 85°C. After 10 hours the solution is loaded on a strong cationic ion exchanger, Biorad AG 50W-X4, and eluted with 6 M ammonia in water containing 50 mM ammonium formate. The solution is evaporated and the title compound is purified by chromatography.

Example 39

3,6,9-Tris-carboxymethyl-4-(5,6-dihydroxy-3-oxa-hexyl)-3,6,9-triazaundecanediacid

(a) 2-(4-(2,2-Dimethyl-1,3-dioxolan-4-yl)-3-oxabutyl)-3-oxo-1,7-bis-triphenylmethyl-1,4,7-triazaheptane



First alternative

2-(2-Hydroxyethyl)-3-oxo-1,7-bis-(triphenylmethyl)-1,4,7-triazaheptane (10 mmol, Example 38(a)) is dissolved in dry tetrahydrofuran (50 ml) and 10 mmol sodium hydride is added. When gas evolution stops at ambient temperature, tetrabutylammonium iodide (1 mmol) and 4-bromomethyl-2,2-dimethyl-1,3-dioxolane (10 mmol) is added. After stirring at ambient temperature for 24 hours, the solvent is evaporated, the product is dissolved in ether, the ether phase is washed with water, dried with sodium sulphate, filtered and the solvent evaporated. The product is purified by chromatography on silica.

Second alternative

The method described by J.M. Lacombe et al, Can. J. Chem., 59 (1981) 473 is employed as follows:

2-(2-Hydroxyethyl)-3-oxo-1,7-bis-(triphenylmethyl)-1,4,7-triazaheptane (10 mmol, Example 38(a)) is dissolved in acetonitrile (or alternatively tetrahydrofuran or dimethylformamide) (50 ml) and cooled to -20°C. Trifluoromethylsulfonic acid anhydride (15 mmol) is added, and then a solution of 2,2-dimethyl-4-hydroxymethyl-1,3-dioxolan (20 mmol) dissolved in the same solvent is added dropwise. After 2 hours at -20°C, the reaction is taken up to ambient temperature and stirred overnight. The solvent is evaporated and the product is purified by chromatography on silica.

(b) 1,5-Diamino-1-(5,6-dihydroxy-3-oxahexyl)-3-azapentane hydrochloride

The product of step (a) is reacted to produce 1,5-diamino-1-(5,6-dihydroxy-3-oxahexyl)-3-azapentane

- 76 -

hydrochloride in a process analogous to that of step (c) of Example 38.

(c) 3,6,9-Triscarboxymethyl-4-(5,6-dihydroxy-3-oxahexyl)-3,6,9-triazaundecanediacid

The product of step (b) is reacted to produce the title compound in a process analogous to that of step (c) of Example 9.

Example 40

Gadolinium (III) chelate of N<sup>3</sup>,N<sup>6</sup>,N<sup>9</sup>-triscarboxymethyl-4,8-bis-hydroxymethyl-3,6,9-triazaundecane diacid disodium salt

(a) Gadolinium (III) chelate of N<sup>3</sup>,N<sup>6</sup>,N<sup>9</sup>-tris-carboxymethyl-4,8-bisbenzyloxymethyl-3,6,9-triazaundecane diacid-disodium salt

N<sup>3</sup>,N<sup>6</sup>,N<sup>9</sup>-Triscarboxymethyl-4,8-bis-benzyloxymethyl-3,6,9-triazaundecane diacid (0.50 g, 0.80 mmol)

(Example 6) in water (10 ml) was added dropwise to a solution of gadolinium chloride (0.28 g, 0.80 mmol) in water (3 ml) at 70°C over 30 minutes. The pH was continuously adjusted with sodium hydroxide (2 M) to be within the range 5-7. The temperature was increased to 85°C for 2.5 hours. Gadolinium (III) chelate of N<sup>3</sup>,N<sup>6</sup>,N<sup>9</sup>-tris-carboxymethyl-4,8-bisbenzyloxymethyl-3,6,9-triazaundecane diacid-disodium salt was isolated by evaporation of the clear solution. Yield 0.81 g (99%) of a clear oil containing sodium chloride (0.14 g, 2.4 mmol).

- 77 -

(b) Gadolinium (III) chelate of  $N^3, N^6, N^9$ -tris-carboxymethyl-4,8-bis(hydroxymethyl)-3,6,9-triazaundecane diacid-disodium salt

To Gadolinium (III) chelate of  $N^3, N^6, N^9$ -tris-carboxymethyl-4,8-bis(benzyloxymethyl)-3,6,9-triazaundecane diacid-disodium salt (0.81 g, 0.79 mmol) (containing 0.14 g sodium chloride) in methanol (25 ml) was added ammonium formate (0.30 g) and 10% Palladium on carbon (0.40 g) at 50°C and the mixture was stirred for 17 hours under a nitrogen atmosphere. The solution was filtered and evaporated. The title compound was isolated as a white powder. Yield 0.56 g (83%) (containing sodium chloride (0.14 g)). M.p. greater than 350°C.

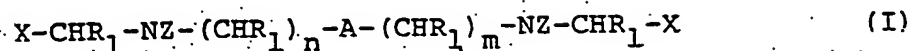
Example 41

Bismuth (III) chelate of 3,6-bis-(carboxymethyl)-4-hydroxyethyl-3,6-diazaoctane diacid mono-sodium-salt

A neutral suspension of bismuth hydroxide was prepared by neutralisation of an acidic solution of bismuth chloride (120 mg, 0.37 mmol, 4 ml) with sodium hydroxide, followed by centrifugation of the precipitate and resuspension of the precipitate in water (4 ml). This solution was added to a neutral solution of 3,6-bis-(carboxymethyl)-4-hydroxyethyl-3,6-diazaoctane diacid (120 mg, 0.37 mmol) (Example 4) in water (4 ml) and the mixture was stirred at 80°C for 16 hours, and refluxed for 4 hours. The clear solution was evaporated, and the title compound was isolated as a white solid. Yield 200 mg (99%). M.p. 250°C.

Claims:

1. A compound of formula I



(wherein each of the groups Z is a group  $-CHR_1X$  or the groups Z together are a group  $-(CHR_1)_q-A'-(CHR_1)_r-$ , where A' is an oxygen or sulphur atom or a group  $-N-Y$ ;

A is a group  $-N-Y$  or  $A-(CHR_1)_m-$  represents a carbon-nitrogen bond or, when the groups Z together are a group  $-(CHR_1)_q-A'-(CHR_1)_r-$ , A may also represent an oxygen or sulphur atom;

each Y, which may be the same or different, is a group  $-(CHR_1)_p-N(CHR_1X)_2$  or a group  $-CHR_1X$ ;

each X, which may be the same or different, is a

carboxyl group or a derivative thereof or a group  $R_1$ ;

each  $R_1$ , which may be the same or different, is

a hydrogen atom, a hydroxyalkyl group or an optionally hydroxylated alkoxy or alkoxyalkyl group;

n, m, p, q and r are each 2, 3 or 4; with the provisos

that at least two nitrogens carry a  $-CHR_1X$  moiety

wherein X is a carboxyl group or a derivative thereof,

that each  $-CHR_1X$  moiety is other than a methyl

group, and that unless A' is oxygen or sulphur

or A is  $N-(CHR_1)_p-N(CHR_1X)_2$  at least one  $R_1$  is

other than hydrogen) or a salt or metal chelate thereof.

2. A compound of formula I as claimed in claim 1

wherein X,  $R_1$ , Z and A are as defined in claim 1

with the further provisos that unless A' is sulphur

or A is  $N-(CHR_1)_p-N(CHR_1X)_2$  at least one  $R_1$  is

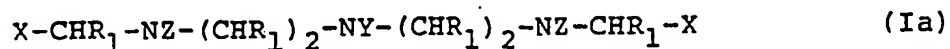
other than hydrogen and that where A and A' are

groups  $N-CHR_1X$  at least one  $CHR_1X$  group is other

- 79 -

than a  $\text{CH}_2\text{X}^3$  group (where  $\text{X}^3$  represents a carboxyl group or a derivative thereof or a 2-hydroxyethyl or 2,3-dihydroxypropyl group), or a salt or metal chelate thereof.

3. A compound as claimed in either of claims 1 and 2 of formula Ia



(wherein each group Z is a group  $-\text{CHR}_1\text{X}$  or the groups Z together are a group  $-(\text{CHR}_1)_2-\text{A}'-(\text{CHR}_1)_2-$ ; each Y, which may be the same or different, is a group  $-(\text{CHR}_1)_2-\text{N}(\text{CHR}_1\text{X})_2$  or  $-\text{CHR}_1\text{X}$ ; and  $\text{A}'$ , X and  $\text{R}_1$  are as defined in claim 1)

or a salt or metal chelate thereof.

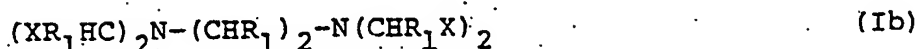
4. A compound as claimed in any one of claims 1 to 3 wherein at least one  $\text{R}_1$  in a moiety  $(\text{CHR}_1)_m$ ,  $(\text{CHR}_1)_n$ ,  $(\text{CHR}_1)_p$ ,  $(\text{CHR}_1)_q$ , or  $(\text{CHR}_1)_r$  (where m, n, p, q, r and  $\text{R}_1$  are as defined in claim 1) is a hydroxyalkyl or optionally hydroxylated alkoxy or alkoxyalkyl group, or a salt or metal chelate thereof.

5. A compound as claimed in any one of claims 1 to 4 wherein the  $\text{R}_1$  moieties, which may be the same or different, are hydrogen or  $\text{C}_{1-8}$  alkoxy, polyalkoxy, hydroxyalkyl, polyhydroxyalkyl, polyhydroxyalkoxy, hydroxyalkoxyalkyl or hydroxypolyalkoxyalkyl groups, or a salt or metal chelate thereof.

6. A compound of formula I as claimed in any one of claims 1 to 5 wherein at least one X moiety is an amide, ester or carboxylate salt group, or a salt or metal chelate thereof.

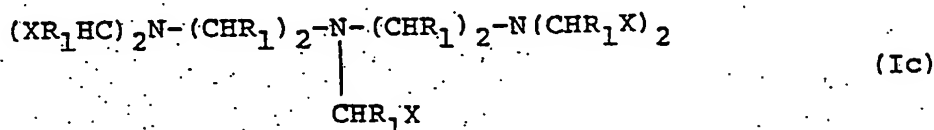
- 80 -

7. A compound as claimed in any one of claims 1 to 6 of formula Ib



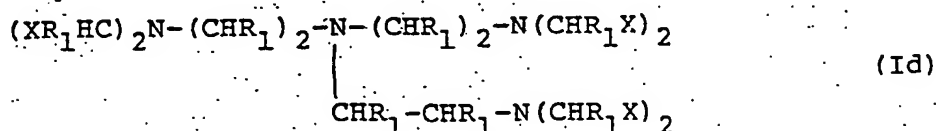
(wherein X and R<sub>1</sub> are as defined in claim 1 and at least two X moieties are ion forming groups), or a salt or metal chelate thereof.

8. A compound as claimed in any one of claims 1 to 6 of formula Ic



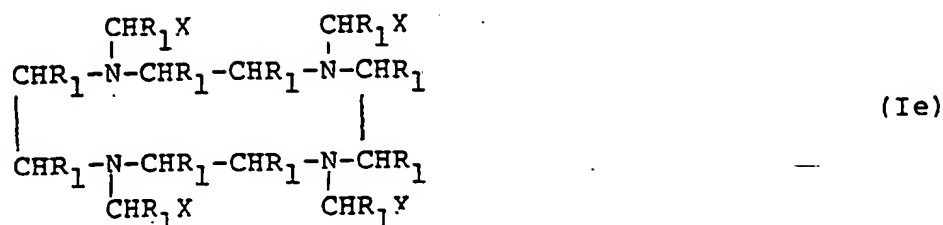
(wherein X and R<sub>1</sub> are as defined in claim 1 and at least two X moieties are ion forming groups), or a salt or metal chelate thereof.

9. A compound as claimed in any one of claims 1 to 6 of formula Id



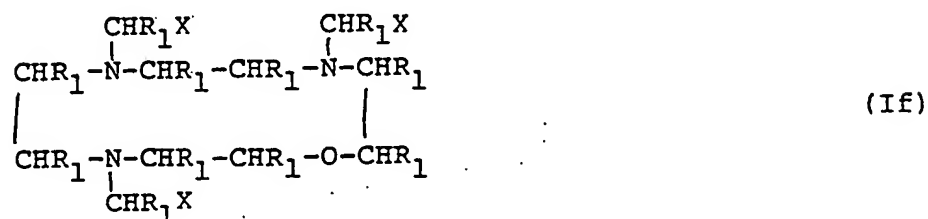
(wherein X and R<sub>1</sub> are as defined in claim 1 and at least two X moieties are ion forming groups), or a salt or metal chelate thereof.

10. A compound as claimed in any one of claims 1 to 6 of formula Ie



(wherein X and R<sub>1</sub> are as defined in claim 1 and at least two X moieties are ion forming groups), or a salt or metal chelate thereof.

11. A compound as claimed in any one of claims 1 to 6 of formula If



(wherein X and R<sub>1</sub> are as defined in claim 1 and at least two X moieties are ion forming groups), or a salt or metal chelate thereof.

12. A compound as claimed in claim 1 being

N-carboxymethyl-N,N-bis-((N'-carboxymethyl-N'-2,3-dihydroxypropyl)-2-aminoethyl)-amine;

N,N,N-tris-((N'-carboxymethyl-N'-2,3-dihydroxypropyl)-2-aminoethyl)-amine;

3,6-bis-(carboxymethyl)-4-hydroxyethyl-3,6-diazaoctanediacid;

1-thia-4,7,10-triazacyclododecane triacetic acid;

N<sup>3</sup>,N<sup>6</sup>,N<sup>9</sup>-tris-carboxymethyl-4,8-bis-hydroxymethyl-3,6,9-triazaundecanediacid;

N<sup>4</sup>,N<sup>7</sup>,N<sup>10</sup>-triscarboxymethyl-1,13-bis-hydroxy-5,9-bis-hydroxymethyl-4,7,10-triazatridecane;

- 82 -

$N^6$ -carboxymethyl- $N^3, N^9$ -bis(methyl carbamylmethyl)-4,8-bishydroxymethyl-3,6,9-triazaundecane diacid;  
3,6,9-tris-carboxymethyl-4-(2-hydroxyethyl)-3,6,9-triazaundecane diacid;  
3,9-bis-(methylcarbamoylmethyl)-6-carboxymethyl-4-(2-hydroxyethyl)-3,6,9-triazaundecanediacid;  
3,6-bis(carboxymethyl)-4-hydroxymethyl-3,6-diazaoctanediacid;  
1-(5-hydroxy-3-oxapentyl)-1,4,7,10-tetraazacyclododecane-4,7,10-triacetic acid;  
1-(8-hydroxy-3,6-dioxaoctyl)-1,4,7,10-tetraazacyclododecane-4,7,10-triacetic acid;  
1-(2-hydroxy-propyl)-1,4,7,10-tetraazacyclododecane-4,7,10-triacetic acid;  
1-(3-hydroxy-propyl)-1,4,7,10-tetraazacyclododecane-4,7,10-triacetic acid;  
5,9-bis(hydroxymethyl)-1-oxa-4,7,10-triazacyclododecane-4,7,10-triacetic acid;  
5,9-bis(hydroxymethyl)-1-thia-4,7,10-triazacyclododecane-4,7,10-triacetic acid;  
 $N^6$ -carboxymethyl- $N^3, N^9$ -bis(2,3-dihydroxypropyl-carbamylmethyl)-4,8-bis(hydroxymethyl)-3,6,9-triazaundecane diacid;  
2-(2-hydroxyethyl)-1,4,7,11-tetrakis-carboxymethyl-1,4,7,11-tetraazacyclododecane;  
3,6,9-triscarboxymethyl-4-(5,6-dihydroxy-3-oxa-hexyl)-3,6,9-triazaundecane diacid;  
or a salt or metal chelate thereof.

13. A compound as claimed in claim 1 being 1-oxa-4,7,10-triazacyclododecane- $N, N', N''$ -triacetic acid, or a salt or metal chelate thereof.

14. A chelate as claimed in any one of claims 1 to 13 being a chelate of a paramagnetic metal species.

15. A diagnostic or therapeutic agent comprising a metal chelate, whereof the chelating entity is

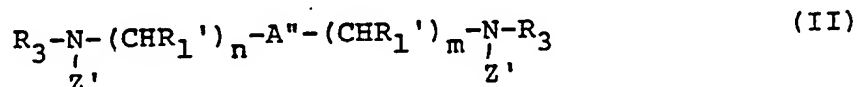


the residue of a compound of formula I as defined in claim 1, together with at least one pharmaceutical or veterinary carrier or excipient.

16. A detoxification agent comprising a chelating agent as claimed in claim 1 in the form of a salt with a physiologically acceptable counterion, together with at least one pharmaceutical or veterinary carrier or excipient.

17. A process for the preparation of compounds as claimed in claim 1, said process comprising at least one of the following steps:

(i) reacting an amine of formula II



(wherein  $R_3$  is a hydrogen atom or a  $-CHR_1'X'$  group;  $X'$  and  $R_1'$  are as defined for  $R_1$  and  $X$  in claim 1 or are groups convertible thereto; each group  $Z'$  is a group  $-CHR_1'X'$  or an  $R_3$  moiety or together the groups  $Z'$  are a  $-(CHR_1')_q-$   $A''-(CHR_1')_r-$  bridging group where  $A''$  is an oxygen or sulphur atom or a group  $-N-Y'$  where  $Y'$  is an  $R_3$  moiety or a group  $-(CHR_1')_p-N(R_3)_2$ ; and  $A''$  is a group  $-N-Y'$  or  $-A''-(CHR_1')_m$  is a carbon-nitrogen bond or, where the groups  $Z'$  together form a bridging group,  $A''$  may also represent an oxygen or sulphur atom; with the provisos that at least one  $R_3$  moiety is a hydrogen atom, that at least two amine nitrogens carry a hydrogen atom or a  $-CHR_1'X'$  moiety in which  $X'$  is convertible to a carboxyl group or a derivative thereof, and that each  $-CHR_1'X'$  moiety is other than a methyl group) to introduce a  $-CHR_1X$  moiety (where  $R_1$  and

- 84 -

X are as defined in claim 1) at an amine nitrogen, followed if necessary by converting  $R'_1$  or X' to  $R_1$  or X;

(ii) converting a carboxyl X moiety in a compound of formula I into a carboxyl derivative or a carboxyl derivative X moiety in a compound of formula I into a carboxyl group; and

(iii) converting a compound of formula I into a salt or metal chelate thereof or converting a salt or chelate of a compound of formula I into a compound of formula I.

18. Use of a compound of formula I or a salt or metal chelate thereof for the manufacture of a diagnostic or therapeutic agent for use in a method of image generation, detoxification or radiotherapy practised on the human or non-human animal body.

19. A method of generating enhanced images of the human or non-human animal body, which method comprises administering to said body a diagnostic agent as claimed in claim 15 and generating an X-ray, MR-diagnostics, ultrasound or scintigraphic image of at least a part thereof.

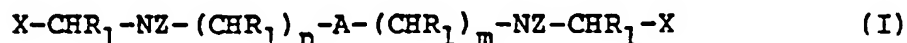
20. A method of radiotherapy practised on the human or non-human animal body, which method comprises administering to said body a chelate of a radioactive metal species, said chelate being a chelate as claimed in claim 1.

21. A method of heavy metal detoxification practised on the human or non-human animal body, which method comprises administering to said body a chelating agent as claimed in claim 1 in the form of a salt with a physiologically acceptable concentration.

## AMENDED CLAIMS

[received by the International Bureau on 17 January 1989 (17.01.89);  
original claim 1 amended; new claims 2 and 3 added; original claims 2-15 renumbered  
as claims 4-17 wherein claim 17 is amended; new claims 18-20 added; original claims 16-21  
renumbered as claims 21-26 (8 pages)]

## 1. A compound of formula I



(wherein each of the groups Z is a group  $-CHR_1X$   
or the groups Z together are a group  $-(CHR_1)_q-A'-(CHR_1)_r-$ , where A' is an oxygen or sulphur atom  
or a group  $-N-Y$ ;

A is a group  $-N-Y$  or  $A-(CHR_1)_m-$  represents a carbon-nitrogen bond or, when the groups Z together are  
a group  $-(CHR_1)_q-A'-(CHR_1)_r-$ , A may also represent  
an oxygen or sulphur atom;

each Y, which may be the same or different, is

a group  $-(CHR_1)_p-N(CHR_1X)_2$  or a group  $-CHR_1X$ ;

each X, which may be the same or different, is a

carboxyl group or a derivative thereof or a group  $R_1$ ;

each  $R_1$ , which may be the same or different, is

a hydrogen atom, a hydroxyalkyl group or an optionally  
hydroxylated alkoxy or alkoxyalkyl group;

n,m,p,q and r are each 2,3 or 4; with the provisos

that at least two nitrogens carry a  $CHR_1X^4$  moiety  
wherein  $X^4$  is a carboxyl group or a derivative thereof,

that each  $CHR_1X$  moiety is other than a methyl group,

that where A is  $NCHR_1X$ , unless A' is S or O, and

where  $A(CHR_1)_m$  is a bond, unless A' is S, at least

one  $R_1$  is other than hydrogen, that where  $(CHR_1)_n$

$A(CHR_1)_m$  is  $(CH_2)_2$  or  $CH_2CH_2NZCH_2CH_2$  and all Z

groups are  $CHR_1X$  then each Z group must represent

other than a group  $CH_2X^3$  where  $X^3$  is a mono or

di-hydroxy  $C_{1-2}$  alkyl group, and that where  $A(CHR_1)_m$

is  $CH_2CHR_1$  and all Z groups are groups  $-CH_2X^4$ , then  $R_1$  is

other than a hydrogen atom or a hydroxymethyl group)

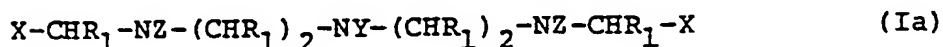
or a salt or metal chelate thereof.

2. A compound of formula I as claimed in claim 1 with the further proviso that unless A' or A is sulphur or both A and A' are oxygen or A is  $N(CH_2R_1)_pN(CHR_1X)_2$  at least one  $R_1$  is other than hydrogen, or a salt or metal chelate of said compound.

3. A compound of formula I as claimed in either of claims 1 and 2 with the further proviso that where  $(CHR_1)_n A (CHR_1)_m$  is  $CH_2CH_2$  or  $CH_2CH_2NZCH_2CH_2$  and all Z groups are  $CHR_1X^4$  then in each group  $CHR_1X^4$   $R_1$  must represent other than a hydroxyalkyl or optionally hydroxylated alkoxyalkyl group, or a salt or metal chelate of a said compound.

4. A compound of formula I as claimed in any one of claims 1 to 3 with the further provisos that unless A' is sulphur or A is  $N-(CHR_1)_pN(CHR_1X)_2$  at least one  $R_1$  is other than hydrogen and that where A and A' are groups  $N-CHR_1X$  at least one  $CHR_1X$  group is other than a  $CH_2X^3$  group (where  $X^3$  represents a carboxyl group or a derivative thereof or a 2-hydroxyethyl or 2,3-dihydroxypropyl group), or a salt or metal chelate thereof.

5. A compound as claimed in any one of claims 1 to 4 of formula Ia



wherein each group Z is a group  $-CHR_1X$  or the groups Z together are a group  $-(CHR_1)_2-A'-(CHR_1)_2-$ ; each Y, which may be the same or different, is a group  $-(CHR_1)_2-N(CHR_1X)_2$  or  $-CHR_1X$ ; and A', X and  $R_1$  are as defined in claim 1) or a salt or metal chelate thereof.

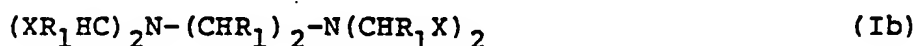
6. A compound as claimed in any one of claims 1 to 5 wherein at least one  $R_1$  in a moiety  $(CHR_1)_m$ ,

$(\text{CHR}_1)_n$ ,  $(\text{CHR}_1)_p$ ,  $(\text{CHR}_1)_q$ , or  $(\text{CHR}_1)_r$  (where  $m$ ,  $n$ ,  $p$ ,  $q$ ,  $r$  and  $R_1$  are as defined in claim 1) is a hydroxyalkyl or optionally hydroxylated alkoxy or alkoxyalkyl group, or a salt or metal chelate thereof.

7. A compound as claimed in any one of claims 1 to 6 wherein the  $R_1$  moieties, which may be the same or different, are hydrogen or  $\text{C}_{1-8}$  alkoxy, polyalkoxy, hydroxyalkyl, polyhydroxyalkyl, polyhydroxyalkoxy, hydroxyalkoxyalkyl or hydroxypolyalkoxyalkyl groups, or a salt or metal chelate thereof.

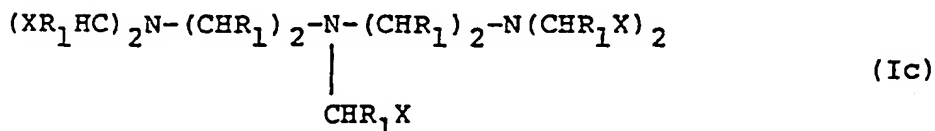
8. A compound of formula I as claimed in any one of claims 1 to 7 wherein at least one X moiety is an amide, ester or carboxylate salt group, or a salt or metal chelate thereof.

9. A compound as claimed in any one of claims 1 to 8 of formula Ib



(wherein X and  $R_1$  are as defined in claim 1 and at least two X moieties are ion forming groups), or a salt or metal chelate thereof.

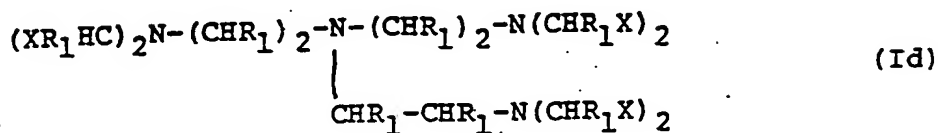
10. A compound as claimed in any one of claims 1 to 8 of formula Ic



(wherein X and  $R_1$  are as defined in claim 1 and at least two X moieties are ion forming groups),

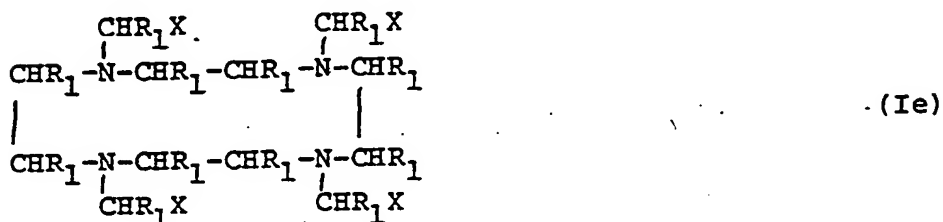
or a salt or metal chelate thereof.

11. A compound as claimed in any one of claims 1 to 8 of formula Id



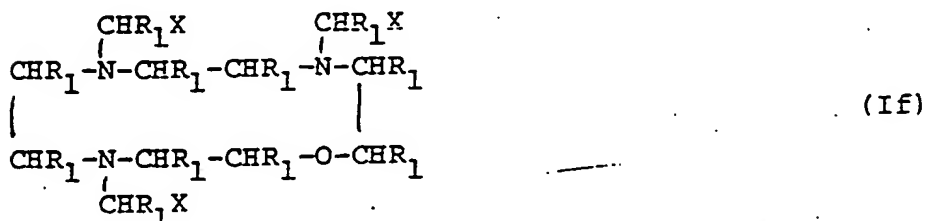
(wherein X and R<sub>1</sub> are as defined in claim 1 and at least two X moieties are ion forming groups), or a salt or metal chelate thereof.

12. A compound as claimed in any one of claims 1 to 8 of formula Ie



(wherein X and R<sub>1</sub> are as defined in claim 1 and at least two X moieties are ion forming groups), or a salt or metal chelate thereof.

13. A compound as claimed in any one of claims 1 to 8 of formula If



(wherein X and R<sub>1</sub> are as defined in claim 1 and

at least two X moieties are ion forming groups),  
or a salt or metal chelate thereof.

14. A compound as claimed in claim 1 being

N-carboxymethyl-N,N-bis-((N'-carboxymethyl-N'-  
2,3-dihydroxypropyl)-2-aminoethyl)-amine;

N,N,N-tris-((N'-carboxymethyl-N'-2,3-dihydroxy-  
propyl)-2-aminoethyl)-amine;

3,6-bis-(carboxymethyl)-4-hydroxyethyl-3,6-diaza-  
octanediacid; .

1-thia-4,7,10-triazacyclododecane triacetic acid;  
N<sup>3</sup>,N<sup>6</sup>,N<sup>9</sup>-tris-carboxymethyl-4,8-bis-hydroxymethyl-  
3,6,9-triazaundecanediacid;

N<sup>4</sup>,N<sup>7</sup>,N<sup>10</sup>-triscarboxymethyl-1,13-bis-hydroxy-5,9-  
bis-hydroxymethyl-4,7,10-triazatridecane;  
N<sup>6</sup>-carboxymethyl-N<sup>3</sup>,N<sup>9</sup>-bis(methyl carbamylmethyl)-  
4,8-bis-hydroxymethyl-3,6,9-triazaundecane diacid;

3,6,9-tris-carboxymethyl-4-(2-hydroxyethyl)-  
3,6,9-triazaundecane diacid;

3,9-bis-(methylcarbamoylmethyl)-6-carboxymethyl-  
4-(2-hydroxyethyl)-3,6,9-triazaundecanediacid;

3,6-bis(carboxymethyl)-4-hydroxymethyl-3,6-  
diazaoctanediacid;

1-(5-hydroxy-3-oxapentyl)-1,4,7,10-tetraazacyclo-  
dodecane-4,7,10-triacetic acid;

1-(8-hydroxy-3,6-dioxaoctyl)-1,4,7,10-tetraaza-  
cyclododecane-4,7,10-triacetic acid;

1-(2-hydroxy-propyl)-1,4,7,10-tetraazacyclodode-  
cane-4,7,10-triacetic acid;

1-(3-hydroxy-propyl)-1,4,7,10-tetraazacyclodode-  
cane-4,7,10-triacetic acid;

5,9-bis(hydroxymethyl)-1-oxa-4,7,10-triazacyclo-  
dodecane-4,7,10-triacetic acid;

5,9-bis(hydroxymethyl)-1-thia-4,7,10-triazacyclo-  
dodecane-4,7,10-triacetic acid;

N<sup>6</sup>-carboxymethyl-N<sup>3</sup>,N<sup>9</sup>-bis(2,3-dihydroxypropyl-  
carbamylmethyl)-4,8-bis(hydroxymethyl)-3,6,9-triaza-

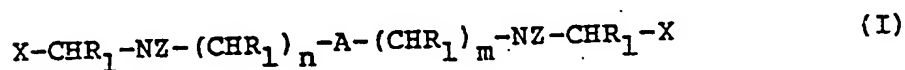
undecane diacid;

2-(2-hydroxyethyl)-1,4,7,11-tetrakis-carboxymethyl-1,4,7,11-tetraazacyclododecane;  
3,6,9-triscarboxymethyl-4-(5,6-dihydroxy-3-oxa-hexyl)-3,6,9-triazaundecane diacid;  
or a salt or metal chelate thereof.

15. A compound as claimed in claim 1 being 1-oxa-4,7,10-triazacyclododecane-N,N',N"-triacetic acid, or a salt or metal chelate thereof.

16. A chelate as claimed in any one of claims 1 to 15 being a chelate of a paramagnetic metal species.

17. A diagnostic or therapeutic agent comprising at least one pharmaceutical or veterinary carrier or excipient together with a metal chelate, whereof the chelating entity is the residue of a compound of formula I



(wherein each of the groups Z is a group  $-CHR_1X$  or the groups Z together are a group  $-(CHR_1)_q-A'-(CHR_1)_r-$ , where A' is an oxygen or sulphur atom or a group  $-N-Y$ ;

A is a group  $-N-Y$  or  $A-(CHR_1)_m-$  represents a carbon-nitrogen bond or, when the groups Z together are a group  $-(CHR_1)_q-A'-(CHR_1)_r-$ , A may also represent an oxygen or sulphur atom;

each Y, which may be the same or different, is

a group  $-(CHR_1)_p-N(CHR_1X)_2$  or a group  $-CHR_1X$ ;

each X, which may be the same or different, is a carboxyl group or a derivative thereof or a group  $R_1$ ;

each  $R_1$ , which may be the same or different, is a hydrogen atom, a hydroxyalkyl group or an optionally hydroxylated alkoxy or alkoxyalkyl group;

n,m,p,q and r are each 2,3 or 4; with the provisos



that at least two nitrogens carry a  $\text{CHR}_1\text{X}^4$  moiety where  $\text{X}^4$  is a carboxyl group or a derivative thereof, that each  $\text{CHR}_1\text{X}$  moiety is other than a methyl group, that unless A is  $\text{N}-(\text{CHR}_1)_p - \text{N}(\text{CHR}_1\text{X})_2$  or is sulphur or oxygen at least one  $\text{R}_1$  is other than hydrogen, and that where  $(\text{CHR}_1)_m \text{A}(\text{CHR}_1)_n$  is  $\text{CH}_2\text{CH}_2\text{N}(\text{CH}_2\text{R}_1)\text{CH}_2\text{CH}_2$  and all other groups Z are  $\text{CH}_2\text{X}^3$ ,  $\text{R}_1$  is other than hydrogen or 2-hydroxy-ethyl) or a salt thereof.

18. An agent as claimed in claim 17 wherein said chelating agent is the residue of a compound of formula I or salt thereof as defined in claim 17 with the further proviso that where  $(\text{CHR}_1)_m \text{A}(\text{CHR}_1)_n$  is  $\text{CH}_2\text{CH}_2$  or  $\text{CH}_2\text{CH}_2\text{NZCH}_2\text{CH}_2$  and all Z groups are  $\text{CHR}_1\text{X}^4$  than each group  $\text{CHR}_1\text{X}^4$   $\text{R}_1$  must represent other than a hydroxyalkyl or optionally hydroxylated alkoxyalkyl group.

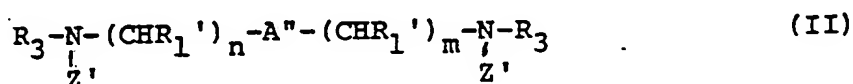
19. An agent as claimed in either of claims 17 and 18 wherein said chelating agent is the residue of a compound of formula I or salt thereof as defined in either of claims 17 and 18 with the further proviso that unless A' or A is sulphur or both A and A' are oxygen or A is  $\text{N}(\text{CH}_2\text{R}_1)_p \text{N}(\text{CHR}_1\text{X})_2$  at least one  $\text{R}_1$  is other than hydrogen.

20. An agent as claimed in claim 17 wherein said chelating agent is the residue of a compound of formula I or salt thereof as defined in any one of claims 1 to 15.

21. A detoxification agent comprising a chelating agent as claimed in any one of claims 1 to 15 in the form of a salt with a physiologically acceptable counterion, together with at least one pharmaceutical or veterinary carrier or excipient.

22. A process for the preparation of compounds as claimed in claim 1, said process comprising at least one of the following steps:

- (i) reacting an amine of formula II



(wherein  $R_3$  is a hydrogen atom or a  $-CHR_1'X'$  group;  $X'$  and  $R_1'$  are as defined for  $R_1$  and  $X$  in claim 1 or are groups convertible thereto; each group  $Z'$  is a group  $-CHR_1'X'$  or an  $R_3$  moiety or together the groups  $Z'$  are a  $-(CHR_1')_q - A'' - (CHR_1')_r -$  bridging group where  $A''$  is an oxygen or sulphur atom or a group  $-N-Y'$  where  $Y'$  is an  $R_3$  moiety or a group  $-(CHR_1')_p - N(R_3)_2$ ; and  $A''$  is a group  $-N-Y'$  or  $-A'' - (CHR_1')_m$  is a carbon-nitrogen bond or, where the groups  $Z'$  together form a bridging group,  $A''$  may also represent an oxygen or sulphur atom; with the provisos that at least one  $R_3$  moiety is a hydrogen atom, that at least two amine nitrogens carry a hydrogen atom or a  $-CHR_1'X'$  moiety in which  $X'$  is convertible to a carboxyl group or a derivative thereof, and that each  $-CHR_1'X'$  moiety is other than a methyl group) to introduce a  $-CHR_1X$  moiety (where  $R_1$  and  $X$  are as defined in claim 1) at an amine nitrogen, followed if necessary by converting  $R_1'$  or  $X'$  to  $R_1$  or  $X$ ;

- (ii) converting a carboxyl  $X$  moiety in a compound of formula I into a carboxyl derivative or a carboxyl derivative  $X$  moiety in a compound of formula I into a carboxyl group; and

- (iii) converting a compound of formula I into a

salt or metal chelate thereof or converting a salt or chelate of a compound of formula I into a compound of formula I.

23. Use of a compound of formula I or a salt or metal chelate thereof as defined in any one of claims 1 to 20 for the manufacture of a diagnostic or therapeutic agent for use in a method of image generation, detoxification or radiotherapy practised on the human or non-human animal body.

24. A method of generating enhanced images of the human or non-human animal body, which method comprises administering to said body a diagnostic agent as claimed in any one of claims 17 to 20 and generating an X-ray, MR-diagnostics, ultrasound or scintigraphic image of at least a part thereof.

25. A method of radiotherapy practised on the human or non-human animal body, which method comprises administering to said body a chelate of a radioactive metal species, said chelate being a chelate as claimed in any one of claims 1 to 16.

26. A method of heavy metal detoxification practised on the human or non-human animal body, which method comprises administering to said body a chelating agent as claimed in any one of claims 1 to 16 in the form of a salt with a physiologically acceptable concentration.

# INTERNATIONAL SEARCH REPORT

International Application No

PCT/GB 88/00572

<b>I. CLASSIFICATION OF SUBJECT MATTER</b> (If several classification symbols apply, indicate all) *		
According to International Patent Classification (IPC) or to both National Classification and IPC		
IPC <sup>4</sup> :	C 07 C 101/26; C 07 D 285/00; C 07 D 273/00; A 61 K 49/00; A 61 K 31/195; A 61 K 31/555	
<b>II. FIELDS SEARCHED</b>		
Minimum Documentation Searched <sup>7</sup>		
Classification System	Classification Symbols	
IPC <sup>4</sup>	C 07 C 101/00; C 07 D 285/00; C 07 D 273/00; A 61 K 49/00; A 61 K 31/00	
Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched <sup>8</sup>		
<b>III. DOCUMENTS CONSIDERED TO BE RELEVANT*</b>		
Category *	Citation of Document, <sup>11</sup> with indication, where appropriate, of the relevant passages <sup>12</sup>	Relevant to Claim No. <sup>13</sup>
X	Chemical Abstracts, volume 104, no. 16, 21 April 1986, (Columbus, Ohio, US), E. Gavin et al.: "A thermometric titrimetric study of the comple- xation of alkaline earth metals by macrocyclic poly(aminocarboxylic) acids", see page 456, abstract 137019y, & J. Chem. Res., Synop. 1985, (11), 334-5	1,2,5,17
X	Chemical Abstracts, volume 73, no. 19, 9 November 1970, (Columbus, Ohio, US), O.I. Samoilova et al.: "Substances with complexing capabilities. XIII. 1-Hydroxy-2,3-diaminopropane- tetraacetic acid", see page 326, abstract 98343u, & Zh. Org. Khim. 1970, 6(8), 1574-7	1-4,5,7, 12,17
X	GB, A, 750481 (THE DOW CHEMICAL CO.) 13 June 1956 see pages 3-7	1-3,17
-- ./. --		
<div style="display: flex; justify-content: space-between;"> <div style="width: 45%;"> <p>* Special categories of cited documents: <sup>10</sup></p> <p>"A" document defining the general state of the art which is not considered to be of particular relevance</p> <p>"E" earlier document but published on or after the international filing date</p> <p>"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</p> <p>"O" document referring to an oral disclosure, use, exhibition or other means</p> <p>"P" document published prior to the international filing date but later than the priority date claimed</p> </div> <div style="width: 45%;"> <p>"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</p> <p>"X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step</p> <p>"Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.</p> <p>"Δ" document member of the same patent family</p> </div> </div>		
<b>IV. CERTIFICATION</b>		
Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report	
14th October 1988	16 NOV 1988	
International Searching Authority	Signature of Authorized Officer	
EUROPEAN PATENT OFFICE	P.C.G. VAN DER PUTTEN	

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)		
Category	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No
X	GB, A, 727483 (THE DOW CHEMICAL CO.) 6 April 1955 see pages 2-4	1-3,5,6, 8,17
X	FR, A, 1144583 (REFINED PRODUCTS CORP.) 15 October 1957 see pages 2-5	1,2,5,7, 17
X	US, A, 2906762 (KNELL) 29 September 1959 see column 1, line 55 - column 4, line 63	1-3,5,6,8, 14,17
X	US, A, 2894023 (RUBIN) 7 July 1959 see the whole document	1,2,5-7, 13-17
X	US, A, 2848469 (KROLL) 19 August 1958 see column 1, line 64 - column 5, line 30	1-3,5,6, 8,14,17
X	US, A, 2831885 (KROLL) 22 April 1958 see column 1, line 70 - column 6, line 9	1-3,5,6, 8,14,17
X	SU, A, 172753 (VASIL'EVA) 7 July 1965 see the whole document	1-3,5,6, 8,14-18
X	DE, A, 3401052 (SCHERING AG) 26 July 1984 see page 53; claims 1-53 cited in the application	1-18
P,X	EP, A, 0230893 (BRACCO INDUSTRIA CHIMICA) 5 August 1987 see pages 7,18-35	1-8,14-18
P,X	EP, A, 0232751 (E.R. SQUIBB & SONS INC.) 19 August 1987 see page 9	1,2,6,11- 18
P,X	EP, A, 0255471 (SCHERING AG) 3 February 1988 see claims 1-12	1,2,5,6, 10,14-18

## FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

V. ☒ OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE <sup>1</sup>

This International search report has not been established in respect of certain claims under Article 17(2) (a) for the following reasons:

1. ☒ Claim numbers ~~\*\*\*~~, because they relate to subject matter not required to be searched by this Authority, namely:

\* \* \* Claims 19-20

See PCT Rule 39.1(iv):

Methods for treatment of the human or animal body by means of surgery or therapy, as well as diagnostic methods.

2. ☐ Claim numbers \_\_\_\_\_, because they relate to parts of the International application that do not comply with the prescribed requirements to such an extent that no meaningful International search can be carried out, specifically:

3. ☐ Claim numbers \_\_\_\_\_, because they are dependent claims and are not drafted in accordance with the second and third sentences of PCT Rule 6.4(a).

VI. ☐ OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING <sup>2</sup>

This International Searching Authority found multiple inventions in this International application as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International search report covers all searchable claims of the International application.

2. ☐ As only some of the required additional search fees were timely paid by the applicant, this International search report covers only those claims of the International application for which fees were paid, specifically claims:

3. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:

4. ☐ As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

## Remark on Protest

- ☐ The additional search fees were accompanied by applicant's protest.  
☐ No protest accompanied the payment of additional search fees.

# ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL PATENT APPLICATION NO.

GB 8800572

SA 23268

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report. The members are as contained in the European Patent Office EDP file on 07/11/88. The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
GB-A- 750481		None	
GB-A- 727483		None	
FR-A- 1144583		None	
US-A- 2906772		None	
US-A- 2894023		None	
US-A- 2848469		None	
US-A- 2831885		None	
SU-A- 172753		None	
DE-A- 3401052	26-07-84	LU-A- 85177 SE-A- 8400254 FR-A, B 2539996 AU-A- 2355984 JP-A- 59139390 GB-A- 2137612 NL-A- 8400079 GB-A, B 2169598 GB-A, B 2169599 US-A- 4647447 CH-B- 660183 FR-A- 2590484 BE-A- 898708 AU-A- 1018488 AU-B- 574658	24-05-84 22-07-84 03-08-84 26-07-84 10-08-84 10-10-84 16-08-84 16-07-86 16-07-86 03-03-87 31-03-87 29-05-87 16-05-84 28-04-88 14-07-88
EP-A- 0230893	05-08-87	AU-A- 6753987 JP-A- 62195388	06-08-87 28-08-87
EP-A- 0232751	19-08-87	JP-A- 62190175	20-08-87
EP-A- 0255471	03-02-88	DE-A- 3625417 AU-A- 7621787	11-02-88 04-02-88

GB 8800572

SA 23268

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report. The members are as contained in the European Patent Office EDP file on 07/11/88  
The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP-A- 0255471		JP-A- 63041468	22-02-88



**This Page is Inserted by IFW Indexing and Scanning  
Operations and is not part of the Official Record**

## **BEST AVAILABLE IMAGES**

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images include but are not limited to the items checked:

- ☐ **BLACK BORDERS**
- ☐ **IMAGE CUT OFF AT TOP, BOTTOM OR SIDES**
- ☐ **FADED TEXT OR DRAWING**
- ☐ **BLURRED OR ILLEGIBLE TEXT OR DRAWING**
- ☐ **SKEWED/SLANTED IMAGES**
- ☐ **COLOR OR BLACK AND WHITE PHOTOGRAPHS**
- ☐ **GRAY SCALE DOCUMENTS**
- ☐ **LINES OR MARKS ON ORIGINAL DOCUMENT**
- ☐ **REFERENCE(S) OR EXHIBIT(S) SUBMITTED ARE POOR QUALITY**
- ☐ **OTHER:** \_\_\_\_\_

**IMAGES ARE BEST AVAILABLE COPY.**

**As rescanning these documents will not correct the image problems checked, please do not report these problems to the IFW Image Problem Mailbox.**